National Aeronautics and Space Administration

Ozone-Induced Foliar Injury

field guide

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www.nasa.gov

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Foreword

NASA Earth Science

from http://nasascience.nasa.gov/

Earth is a complex, dynamic system we do not yet fully understand. The Earth system, like the human body, comprises diverse components that interact in complex ways. We need to understand the Earth's atmosphere, lithosphere, hydrosphere, cryosphere, and biosphere as a single connected system. Our planet is changing on all spatial and temporal scales. The purpose of NASA's Earth science program is to develop a scientific understanding of Earth's system and its response to natural or human-induced changes, and to improve prediction of climate, weather, and natural hazards.

NASA recently completed deployment of the Earth Observing System, the world's most advanced and comprehensive capability to measure global climate change. Over the coming decade, NASA and the Agency's research partners will be analyzing EOS data to characterize, understand, and predict variability and trends in Earth's system for both research and applications. Earth is the only planet we know to be capable of sustaining life. It is our lifeboat in the vast expanse of space. Over the past 50 years, world population has doubled, grain yields have tripled and economic output has grown sevenfold. Earth science research can ascertain whether and how the Earth can sustain this growth in the future. Also, over a third of the U.S. economy—$3 trillion annually—is influenced by climate, weather, space weather, and natural hazards, providing economic incentive to study the Earth.

NASA Earth System Science conducts and sponsors research, collects new observations from space, develops technologies and extends science and technology education to learners of all ages. We work closely with our global partners in government, industry, and the public to enhance economic security, and environmental stewardship, benefiting society in many tangible ways. We conduct and sponsor research to answer fundamental science questions about the changes we see in climate, weather, and natural hazards, and deliver sound science that helps decision-makers make informed decisions. We inspire the next generation of explorers by providing opportunities for learners of all ages to investigate the Earth system using unique NASA resources, and our Earth System research is strengthening science, technology, engineering and mathematics education nationwide. This is a fundamental part of our mission because the leaders and citizens who will meet challenges of tomorrow are the students of today.

Satellite Data for Earth Science Studies

from http://science.hq.nasa.gov/

NASA's current satellite missions have provided valuable detailed global data that research scientists have used to create models of the chemistry and dynamics of Earth's atmosphere. Using these data, Dr. Jack Fishman, a senior scientist with NASA Langley Research Center's Science Directorate, has been involved in the complex process of determining air pollution sources, pollution transport patterns and ozone air pollution influence on climate change and vegetation. Fishman gathered information for his research from the resources available to him through NASA satellites that predated the current EOS.

In 1978, NASA launched the Total Ozone Mapping Spectrometer (TOMS) aboard the Nimbus 7 satellite, thereby providing daily global measurements of total ozone (i.e., all the ozone in both the lower atmosphere and the stratosphere). In 1979, the Stratospheric Aerosol and Gas Experiment (SAGE) was launched on the Applications Explorer Mission-B satellite and provided profiles of ozone distribution in the stratosphere. Although the objective of both instruments was to provide information about the distribution of stratospheric ozone, where approximately 90 percent of the Earth's ozone resides, the difference in how measurements were made allowed Fishman to provide an indirect measurement of the amount of ozone in the lower atmosphere (Fishman et al., 1990; 2003).

Subsequently, several copies of both the TOMS and SAGE instruments provided data until 2005. In 2004, NASA launched the Aura satellite as part of EOS, and the European Space Agency also has launched several satellites that provide the same measurement capability as did the original TOMS and SAGE instruments three decades earlier.

NASA's satellites include Earth observations and models that use chlorophyll concentrations to indicate the distribution and abundance of vegetation as well as the national distribution and levels of ozone air pollution.

NASA Researchers and Educators Partner

Implementing a Local Ozone Bioindicator Garden to Raise Environmental Awareness

In addition to studying ozone in the lower atmosphere exclusively for research purposes, Dr. Fishman believed that the global spread of ozone pollution was just as important a global-change issue as global warming or stratospheric ozone depletion. To spread the message to the general public, he wrote the book *Global Alert: The Ozone Pollution Crisis* with journalist Robert Kalish in 1990 and thought to expand his message by developing a program that would get children and teachers excited about studying ozone air pollution. In 1998, he wrote a proposal to the Global Learning and Observations to Benefit the Environment (GLOBE) initiative, which was established in 1994 through a consortium of U.S. federal agencies. The proposed Surface Ozone Project for GLOBE provided a means by which an international education program could be developed using a hand-held optical scanner, ozone chemically sensitive strips, and protocols designed to collect information on ozone and supporting measurements for submission to the GLOBE Student Data Server (www.globe.gov). GLOBE inherently included learning possibilities such as formulating questions, calibrating and using scientific instruments, and gathering data to answer local questions through both formal and informal educational settings.

A critical component of the GLOBE project was the establishment of an educator/science team; Dr. Irene Ladd, a veteran teacher in the Governor Wentworth Regional School District, Wolfeboro Falls, New Hampshire, led this aspect of the project as the education co-principal investigator. As part of the project's continuation, a second proposal was submitted to GLOBE in 2002, and Dr. Margaret Pippin, NASA's Langley Research Center, Hampton, Virginia, and Dr. Linda Bush, Knox College, Galesburg, Illinois, joined the team as co-investigators, allowing for improved accuracy of student measurements of ambient ozone concentrations using the hand-held optical scanner.

With her vast experience as a classroom teacher, Dr. Ladd knew that it was important to develop an interactive visual activity that supported measuring an invisible gas, ozone air pollution. Involving students and community members in observing the presence of ozone air pollution by monitoring ozone-induced foliar injury on sensitive plants, called bioindicators, was a viable extension. Through formal and informal education programs, students and community members would be able to monitor ozone air pollution levels as well as observe and record ozone-induced foliar injury on selected ozone-sensitive plants within "bioindicator gardens." The investigation would demonstrate the delicate relationship of air quality to the health of vegetation. The importance of ensuring that both formal and informal learners receive accurate guidance on how to implement an ozone bioindicator garden for the purpose of monitoring ozone-induced injury was then identified. From this need, the team set forth plans for the development of a field guide and accompanying training mechanisms. The program outlined in this implementation guide is the result of those efforts.

Section I

Surface Ozone Air Pollution

Introduction to Ozone Air Pollution

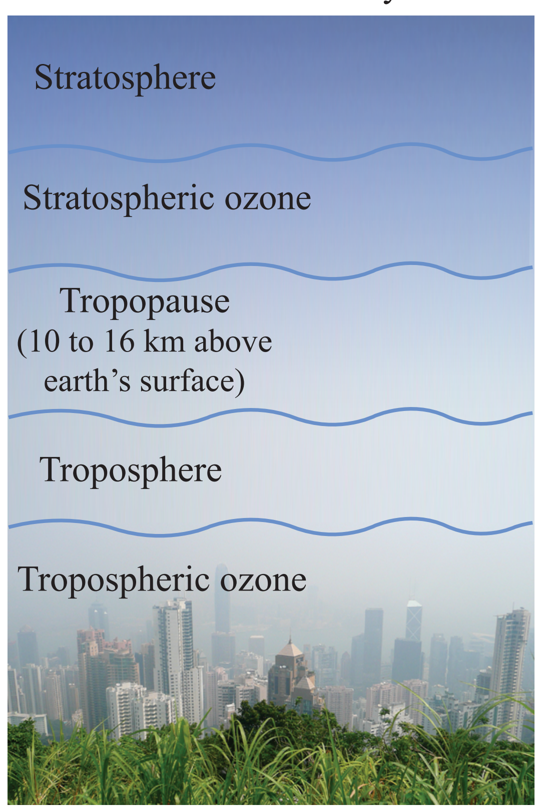
Atmospheric Composition. The atmosphere is made up of a mixture of gases. The gases include molecular nitrogen (N_2) and oxygen (O_2), and carbon dioxide (CO_2), water vapor (H_2O), argon, and trace gases. Nitrogen makes up approximately 78 percent of the atmosphere and oxygen 21 percent. Only about 1 percent of the atmosphere is made up of a combination of the remaining gases, and collectively these gases are commonly referred to as trace gases.

Air quality is affected by the atmosphere's composition. The addition of pollutants created from various human activities gradually contributes to air quality deterioration. Studying surface ozone air pollution (ozone produced near the ground where people breathe) and using ozone sensitive plants, or those that show ozone-induced injury, as bioindicators will help develop an understanding of how human behavior affects air quality and foster an awareness of its environmental impact.

Good Versus Bad Ozone. Ozone is a colorless, highly reactive gas that is both a natural component of the atmosphere and a pollutant. In its naturally occurring state in the stratosphere, ozone protects Earth from too much of the radiation. When it is present in excessive quantities near Earth's surface, ozone is a pollutant. Normally the high levels of ozone associated with smog reside in the lowest 2 to 3 km of the atmosphere.

Earth's Atmosphere

The First Two Layers

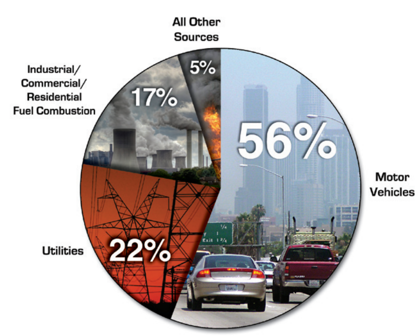


The word ozone comes from the Greek *ozein*, which means “to smell.” The name probably originated from early laboratory studies when ozone was first discovered because of its distinctive acrid odor. While a professor at the University of Basel in Switzerland, the German scientist Christian Friedrich Schönbein is credited with ozone’s discovery in 1839. One of Schönbein’s research goals was to show that ozone is a permanent and natural component of the atmosphere. He devised a method to measure ozone in the atmosphere that was capable of detecting very low values simply and easily. The method used soon became known as Schönbein paper and involved the simple process of saturating a strip of paper with potassium iodide (KI), a common chemical compound often found in a standard chemistry lab, and then allowing it to dry. When placed in the presence of ozone, the potassium iodide oxidized and was converted to potassium iodate (KIO_3). In the conversion process, the paper changed color to various hues of blue. More ozone present in the atmosphere resulted in the paper becoming a deeper shade of blue. Schönbein calibrated the amount of color change following an 8-hour exposure into a measurement standard called Schönbein units, which still today allows scientists to put out a new piece of Schönbein paper each day and measure the relative amount of ozone in the atmosphere.

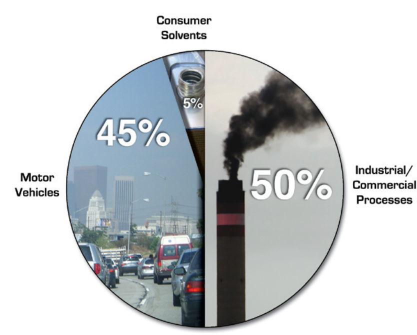
In the early part of the 20th century, ground-based and balloon-borne measurements revealed that most atmospheric ozone is located in the stratosphere with highest concentrations located between altitudes of 15 and 30 km. For a long time, it was believed that tropospheric ozone (or ozone in the lower atmosphere) originated from the stratosphere and that most of it was destroyed by contact with Earth’s surface. Ozone was known to be produced by the splitting of molecular oxygen, O_2, into its two atoms, a process that can only be initiated by strong ultraviolet radiation that does not penetrate past the very high air of the stratosphere. The atomic oxygen formed as a product of this process, called photodissociation, would then recombine with another oxygen molecule to make ozone (hence the O_3 designation). Because the short-wavelength radiation necessary to this process is present only in the stratosphere, no tropospheric ozone production is possible by this mechanism. Additionally, stratospheric ozone absorbs ultraviolet radiation, keeping harmful amounts from reaching Earth’s surface. If such radiation did reach the Earth’s surface, many detrimental effects would occur, including intense sunburn, cataracts, and increased incidence of skin cancer. For this reason, ozone in the stratosphere is often referred to as “good” ozone.

In the 1940s, it became obvious that production of ozone was also taking place in the troposphere. The overall reaction mechanism was eventually identified by Arie Haagen-Smit of the California Institute of Technology. Studies conducted in the highly polluted air of southern California clearly demonstrated that ozone was the major component of greatest concern in photochemical smog, and has subsequently been referred to as "bad" ozone. The smog chemistry hypothesized by Haagen-Smit was still thought to be a relatively small source on the global scale since approximately 90 percent of the ozone was located in the stratosphere, creating a ubiquitous source of tropospheric ozone as air from the stratosphere was transported downward into the troposphere. It wasn't until the 1970s that this viewpoint was challenged when Paul Crutzen, and other scientists at the time, showed that consideration of "smog chemistry" in the background troposphere could produce a sizable source of tropospheric ozone and must be included in the global tropospheric ozone budget. Crutzen's pioneering work on tropospheric ozone was noted when he received the Nobel Prize for Chemistry in 1995 (Crutzen, 1995).

Ozone Pollution Cycle. Human activity has indeed added concentrations of pollutants to the air we breathe. The U.S. Environmental Protection Agency (EPA) AirNow identifies the primary manmade sources of air pollution to be vehicles (automobiles, trucks, buses and airplanes) and industrial burning of fossil fuels (oil/coal burning utility plants and other industrial sources). The primary gases contributing to production of surface ozone are found in the exhaust from any internal combustion engine. Major byproducts of the combustion process are: nitrogen oxides ($NOx = NO + NO_2$), carbon monoxide (CO), and partially burned volatile organic compounds (VOCs, hydrocarbons), which are very reactive.



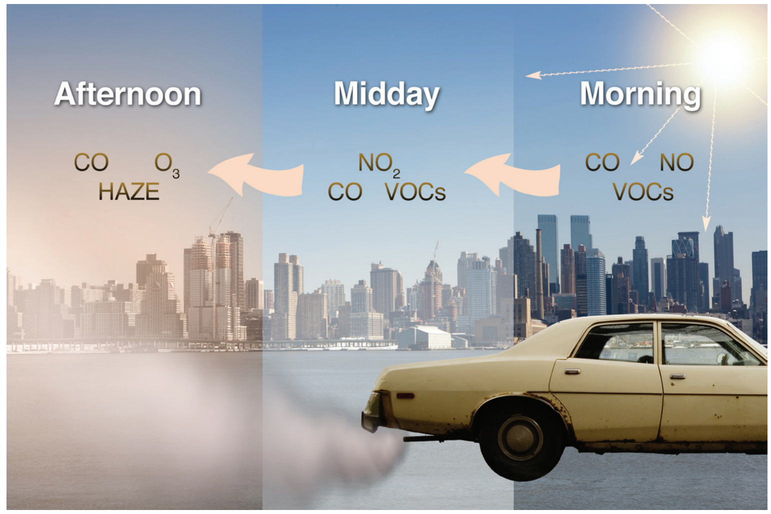
Sources of Anthropogenic NOx

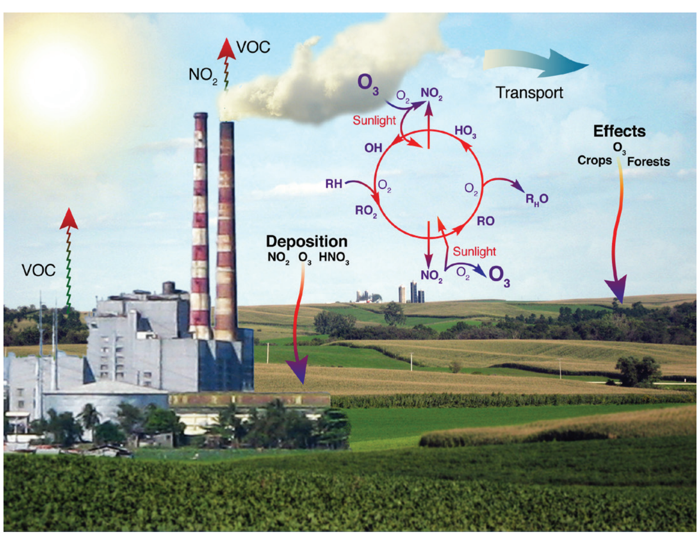


Sources of Anthropogenic VOC

Nitrogen and oxygen molecules in the atmosphere are nonreactive to the sun's energy (they don't split into atoms and attach to other atoms to form something new), but the intense heat within an engine's combustion chamber causes the nitrogen and oxygen molecules to split into nitrogen and oxygen atoms. The split nitrogen atoms link up with oxygen atoms and form a byproduct called nitric oxide (NO).

The air taken into the engine combines with the gasoline (hydrocarbons) before entering the combustion cylinders. If there were complete combustion, as the hydrocarbon or gasoline molecules ignite and split and produce energy in the engine, the byproducts would simply be carbon dioxide (CO_2) and water vapor. However, complete combustion does not occur and some hydrocarbons are exhausted and remain in the troposphere.





The new gases created from human activity (anthropogenic) have contributed to production of higher concentrations of surface ozone (i.e., the formation of ozone air pollution). In general, ozone is a main component of urban smog, and it is commonly referred to as photochemical smog. The production of surface ozone air pollution is dependent upon the sun's energy and occurs faster on bright sunny days with high temperatures. Thus, ozone concentrations tend to increasingly develop from morning to the afternoon. Throughout the day, pollutants react in the presence of the sun's radiant energy and produce a photochemically formed hazy air mass (smog) that may have a brown tinge due to unburned carbonaceous materials (particulate matter) and oxides of nitrogen. At night, the photochemical production of ozone shuts down, and there are nighttime losses due to other chemicals in the atmosphere reacting with ozone. That is why each day may begin with a lower concentration of ozone air pollution than the preceding afternoon.

Image STS092-713-32_2 courtesy of Earth Sciences and Image Analysis Laboratory, NASA Johnson Space Center

Atmospheric Inversions. Radiational or thermal inversions commonly occur in mountainous terrain during late spring, through summer, and into late fall. As the sun sets behind the taller mountains, the most recently warmed air mass in the valleys rises as an intact parcel of air. The cooler, and therefore heavier, air from the mountaintops literally rushes down the slopes to fill the valley with cooler air. Two phenomena occur: 1) the warm air mass rises as a parcel from the valley floors, and 2) as it encounters the cooler air aloft of the mountaintops it is quickly cooled, stops rising, and then stagnates as a layer at mountaintop and or at high-slope heights.

If there are major sources of air pollution in the valleys, this layered air mass likely contains high concentrations of ozone that will interact with plants during the late evening and early morning sunlit hours before dissipating during the next day's cycle. At the valley bottoms, during the actual inversion, the cool air as it arrives causes a build-up of pollution from industrial and vehicle exhaust due to rapid cooling of the exhausted gases and no vertical air mass rise out of the valley during the nighttime inversion. This polluted air mass then rises up and out of the valley with warming of the valley air during the next morning's sunrise. Thus during inversions, vegetation on mountaintops may actually receive higher ozone exposures with significantly more injury occurring to sensitive species.

The astronauts on space shuttle flight STS-92 captured a view of upstate New York at sunset on October 21, 2000. A regional smog layer extended across central New York, western Lake Erie, and Ohio. An atmospheric inversion trapped the pollution layer. The atmospheric inversion is capped by the layer of clouds at the top of the photograph.

Ozone Air Pollution is Harmful. High concentrations of ozone air pollution are harmful to both animals and plants. Concern for poor air quality is reflected most during hot summer days as "ozone alert days," or some other phrase used to warn citizens to restrict outside activity due to the likelihood of higher exposures to ozone. Why? Because exposure to elevated concentrations of surface ozone over extended periods of time causes health problems. Ozone damages the immune system's defenses, making one susceptible to lung infections. Ozone also causes acute respiratory irritation, breathing problems, and aggravates asthma. The pollutant decreases lung capacity by anywhere from 15 to more than 20 percent in sensitive individuals.

Children and the elderly are at greatest risk from surface ozone exposure. Children spend more time outside involved in vigorous activities and have a greater demand for intake of air. Their respiratory systems are developing and are most susceptible to permanent damage. The elderly are also more sensitive to ozone because their immune systems and breathing capabilities are not as strong as when they were younger.

A fact sheet on the effects of ozone air pollution, "The State of the Air 2007," a report on the dangers of ozone air pollution to humans, is electronically available on American Lung Association Website www.lungusa.org

Plants are not unlike humans, but the opposite process takes place. Whereas humans take in oxygen and give off carbon dioxide, plants need to take in carbon dioxide for photosynthesis, the process they use to produce their own food and give off oxygen as a byproduct. Healthy plants, like young children, are actively" taking in" the air. Along with carbon dioxide, ozone passively enters leaves through their stomata. Stomata are small pores, usually on the underside of a leaf, that allow gases to enter or leave. In the presence of sunlight and water the stomata are open and carbon dioxide enters. The plant uses the carbon dioxide to make its food, such as sugars and starches in the all-important process know as photosynthesis, but at the same time, water exits the leaf through the process known as transpiration. If ozone is present in the air, it too will enter the leaf through the open stomata, following much the same path as the carbon dioxide.

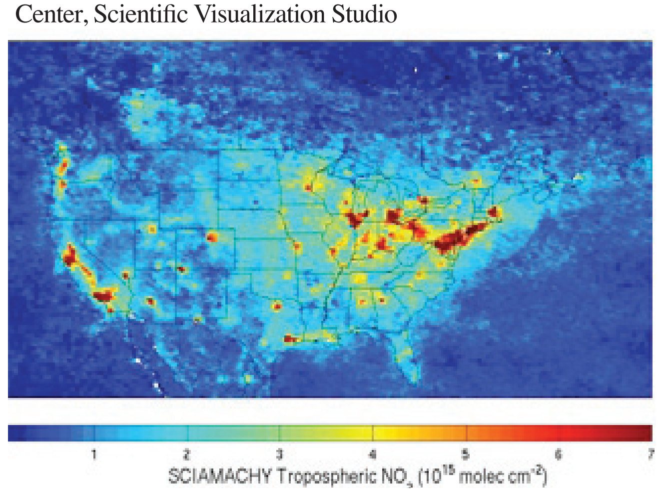
Ozone interferes with a plant's ability to produce and store food. It weakens the plant, making it less resistant to disease and insect infestations. In some sensitive agricultural crops, such as varieties of beans, exposure to ozone air pollution also affects the plant's ability to reproduce, thus decreasing crop yield (e.g., bean production size and numbers are reduced).

Variability of Ozone Levels. Ozone air pollution concentrations in the ambient air can vary within the course of a single day, month, or year, and in geographic areas or regions near one another. Concentrations of ozone air pollution begin to increase from about April through September in the Northern Hemisphere. This is the time period of increased amounts of sunlight, higher temperatures, and commonly occurring stagnating high-pressure systems (Bermuda Highs) over vast regions of the Midwest and Mid-Atlantic regions of the U.S. Under these atmospheric conditions ozone air pollution reaches its highest levels during the hottest and sunniest months of the year. Depending upon weather patterns, the concentrations of ozone air pollution, though usually higher in the summer, can vary year to year. For example, during the hot, dry years, ozone can reach high, unhealthy values and during a wet, cool year, ozone can be greatly reduced to near naturally occurring background concentrations.

Ozone Transport. During the 1950s and 1960s it was believed that surface ozone was a local problem existing in southern California with Los Angeles being most affected by the production of photochemical smog. Human activities create the primary precursor pollutants (NOx and VOCs). The precursor pollutants that lead to regional scale ozone formation are emitted from densely populated areas. Transport of the resulting ozone air pollution occurs across large regions of rural, agricultural, and forested regions of the country.

After the passage of the Clean Air Act in 1970, the EPA set up many more monitoring stations across the U.S. The monitoring system continues to show increased pollution-laden air

This image of human-made lights highlights the most populated ares in the U.S. NASA Goddard Space Flight Center, Scientific Visualization Studio

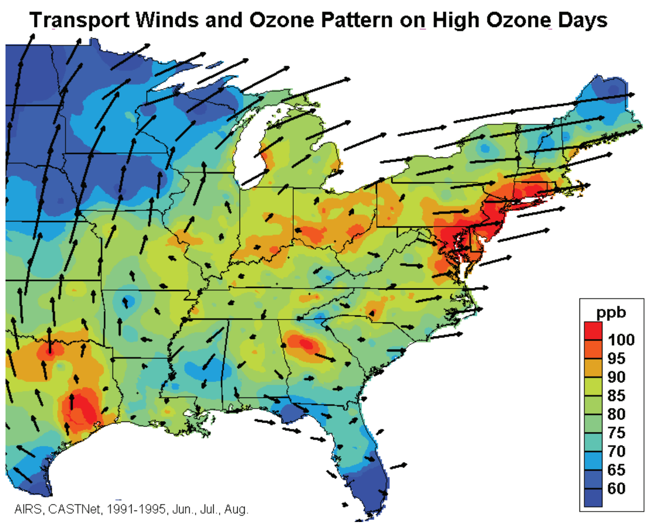


The above satelite image from the climatology year 2003 identifies the location of industrial emissions in the U.S.

masses traveling well beyond the local areas where ozone and its photochemical precursor pollutants are initially produced. The slow moving air masses accumulate pollutants all day long as they travel over industrial centers, large fossil fuel-fired power plants, incinerators, and more importantly, over large and even small urban areas with many forms of fossil fuel-fired transportation. The collection of pollutant gases in slow-moving air masses quickly reacts in the warm sunlit air, and the most important air pollutant formed within these air masses is ozone air pollution.

The series of photographs showing Mount Washington, New Hampshire are from CAMNET and are indicative of visibility impairment occurring during air stagnations (http://hazecam.net). Slow moving high-pressure air masses are common occurrences in the eastern U.S. These air masses transport significant concentrations of ozone air pollution to the plant communities of mountainous, forested, agricultural, and rural areas downwind of the industrial urban areas.

Slow moving air masses allow pollutants to "cook" all day long. As they meander, even more ozone can be formed as the air masses pass over even relatively small urban areas. This complicated chemistry and meteorology can result in a stagnating air mass engulfing a site with smog. This polluted air mass may also be transported from its source to other surrounding areas, which may include pristine wilderness areas and national parks. Our wilderness areas and national parks are natural and cultural landmarks of our country and provide unique visual, ecological, economic, and social value. Mandates have been passed to protect them, but the veil of haze containing ozone air pollution continues to be found lingering over these areas causing injury to the plants, diminished visibility, and impacting visitors' experiences.



The EPA has established AIRS CASTNet, a network set up for long term emissions monitoring and tracking. The acquired EPA data are used to design air quality visualization models of transport winds and ozone pollutants on high ozone days as illustrated on the map. The model covers the analysis of ozone and meteorological data from 1991-1995.

Depending upon the meteorological situation, high levels of ozone can remain over an area for an extended period of time. Plants will be exposed to ozone air pollution and sensitive plants, such as black cherry, will develop ozone-induced symptoms. The injury may weaken a tree, affects its overall health, and make it susceptible to disease, insects, and extreme weather.

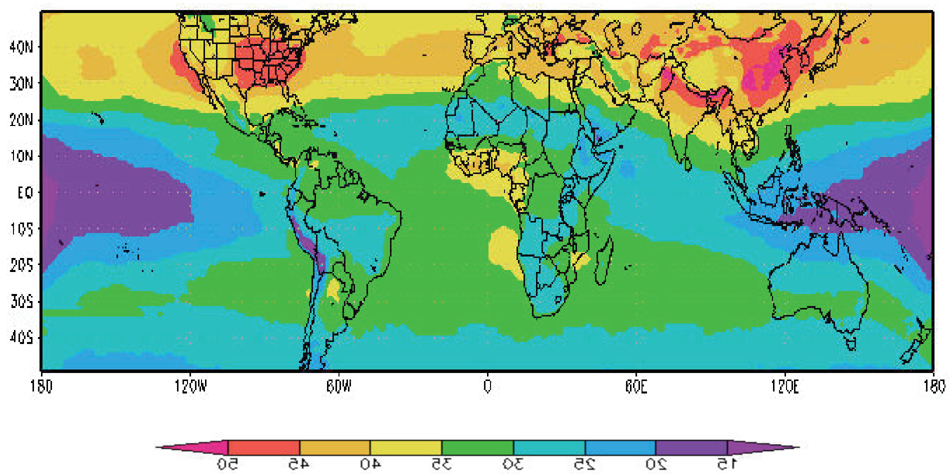
Leaves of the Black Cherry. Other sensitive plants injured by ozone may be found in several Web sites listed in the appendix as resources

Global Satellite Research. Our planet Earth comprises systems that interact in very complex ways. There is a need to understand how Earth's atmosphere interacts with these systems and how our planet is changing. NASA satellites take comprehensive measurements of atmospheric trace gases and provide global visualizations of Earth's atmosphere over time. Many of the satellites are equipped with multiple instruments to provide data that answer questions about ozone trends, air quality changes, and their links to climate change. These instruments map global source regions of ozone pollution.

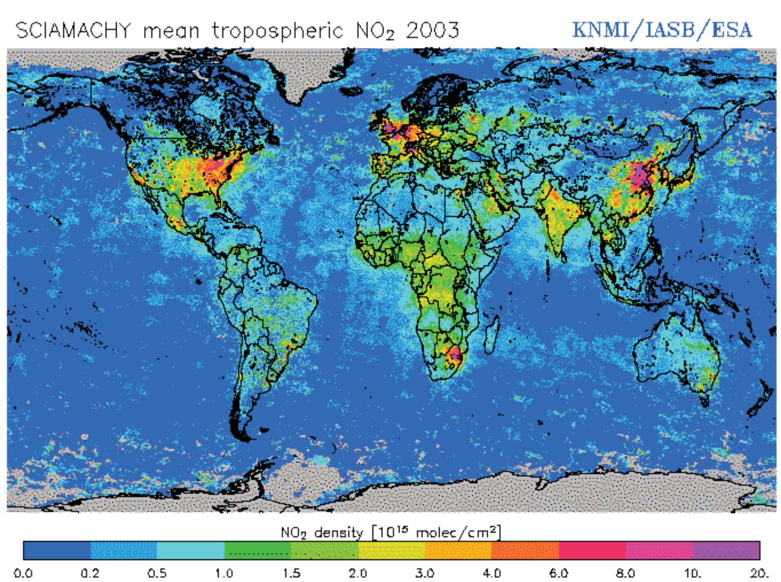
Tropospheric ozone regional "hotspots" were first identified using archived measurements from satellites designed to study the stratosphere (Fishman et al., 1990; 2003). The quantities depicted in this figure reflect the amount of ozone between Earth's surface and the tropopause. Although there is not a straightforward conversion between Dobson units (DU) and ppb at the surface, one study suggests that a DU is approximately equal to 1.2 ppb during summertime conditions (Fishman 2008). Measurements of NO_2 from newer satellites (SCIAMACHY, 2003) confirm the regional nature of pollution drives global pollution distribution more than a decade later.

In the summertime, high ozone concentrations are now found over China and India, where pollution controls are not as rigid as in the U.S. Scientists also believe that emissions from rapidly developing counties will continue to increase. The valuable satellite data can be used to design predictive models and information for local and national government agencies.

Satellite Tropospheric Ozone (June-August)



Tropospheric Ozone (Dobson



Importance of Monitoring Ground Level Ozone Presence

Surface Monitoring Equipment. The EPA monitoring network provides ground-level ozone air pollution data from regions across the U.S. EPA data are available in various formats during those months of increased ozone presence (about May through October) depending upon the reporting model for environmental quality used within a given state. Many state sites post hourly ozone levels on state Web sites, and diurnal data for several days may be requested from EPA and nearby state offices.

Usefulness of Bioindicators. Many highly sensitive plant species have been evaluated for their potential use as bioindicator species capable of detecting the presence of ozone air pollution through the development of very specific and distinctive foliar symptoms. Several species have been extensively researched and are now available for planting in easily developed bioindicator gardens. The species will most likely respond to ozone exposures by exhibiting very unique symptoms, as depicted in subsequent sections discussing the specific species this guide includes.

The U.S. Department of Agriculture Forest Service (USDAFS), Forest Inventory and Analysis Section, uses ozone sensitive species within open sites of the forests as ozone bioindicators to provide information needed to assess the health of America's forests (http://www.fia.fs.fed.us/library/fact-sheets/p3-factsheets/Ozone.pdf). However, there are still large areas within the U.S. without ground-level ozone monitoring equipment.

Biological indicators can provide a much needed tool to detect the presence of ozone pollution in areas that are not currently being monitored, and they provide additional information about the impacts of ozone in areas that are not monitored. Well trained individuals or groups are encouraged to concurrently observe and record ozone-induced plant injury using this guide, and to measure the presence of ozone air pollution using the protocol and instrument designed by the NASA Langley Science Educator Team for the GLOBE program.

Ozone Affects Plants and Causes Economic Loss

Ozone air pollution has been known since the late 1950s to cause significant injury and economic losses to many agricultural crops, herbaceous ornamentals, native plants and numerous forest tree species throughout many regions of the U.S., Canada, and Mexico. First discoveries of direct effects included confirmation of ozone-caused symptoms on grapes in California followed by similar confirmations of symptoms on certain varieties of tobacco, potato, beans, and eastern white pine in eastern U.S. (Karnosky et al., 2007). Many agricultural, forest, and native plant species are continuing to be identified as sensitive to ozone air pollution with confirmation of field symptoms being successfully duplicated under controlled ozone exposures within laboratory and field chamber investigations (Burkey et al., 2005; Innes et al., 2001; Lee et al., 2008; Orendovici et al., 2003).

Foliar symptoms and related productivity effects have been documented on ozone-sensitive plant species in many European and Asiatic countries. Given projected trends in populations, economic outputs, and the associated increased demands for required energy supplies, the impacts of ozone air pollution are very likely to increase.

Criteria for Bioindicator Species. Using biological indicators to detect the presence of ozone through observing levels of plant injury is a long standing effective methodology. A bioindicator can be defined as a vascular or nonvascular organism (in this case, plants) exhibiting a typical and verifiable response when exposed to a specific stressor, such as excessive ozone air pollution.

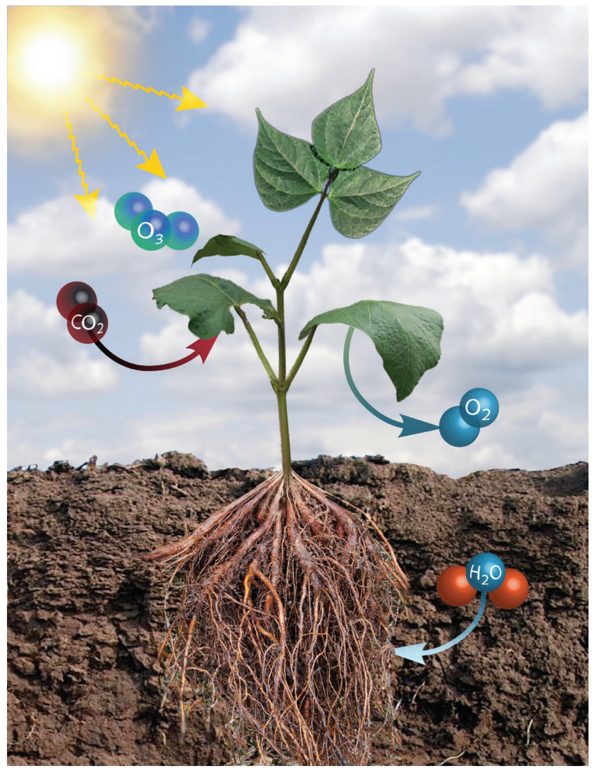
Criteria for a "Good" Bioindicator

- **Easily found across wide geographic range**
- **Grows in diverse habitats**
- **Easily recognized and has smaller sized plants within its population**
- **Has specific proven symptoms appear when exposed to ozone**
- **Displays a consistent, increasing response to elevating ozone exposure**
- **Is genetically stable, with no or very few variants**
- **Is free of any major insect pests and disease causing pathogens**

These sensitive plants can be used to detect the presence of ozone air pollution at a specific location or region and can provide unique information regarding changes in air quality. This is accomplished by observing the changes in ratio of injured plants to noninjured plants, as well as the amount and severity of ozone-induced foliar injury to a single plant. For the purpose of the ozone bioindicator garden, plant indicators of ozone injury can be either introduced (tolerant and sensitive snap beans) or native species (plant species such as common and tall milkweed and cutleaf coneflower). The snap bean, milkweed, and cutleaf coneflower have been used repeatedly in controlled and field research to determine the effects of ozone air pollution. These ozone-sensitive species provide excellent indicators in local areas with validated responses to ambient ozone conditions.

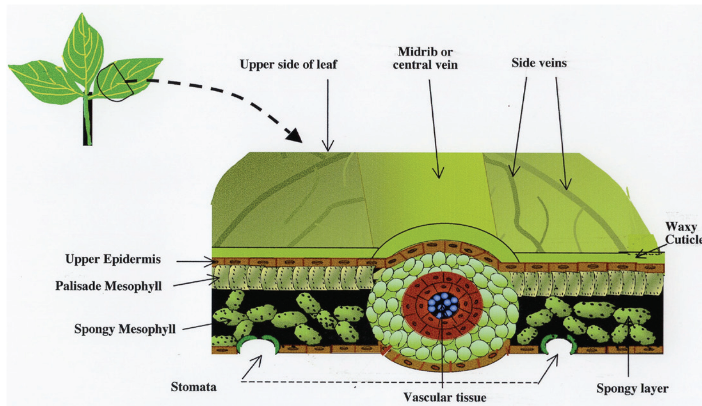
Photosynthesis in Broad Leaf Plants Affected by Ozone Air Pollution

Photosynthesis. Plants are photosynthetic organisms, meaning they function by taking water and nutrients from the soil, light driven energy from the sun, and carbon dioxide (CO_2) from the air for food production, which is needed in order to maintain physiological activity. Plant roots absorb water and nutrients through tiny hairs that extend into the soil. The water carries those nutrients up the main stem and out to all of a plant's leaves. The leaf's stomata "take in" CO_2 from the air and sunlight is absorbed through upper leaf surfaces. With sun exposure acting as energy, CO_2 from the air and nutrients from the soil, or raw materials, are processed into sugars for the plant's consumption.



During photosynthesis, plants simultaneously give off oxygen and lose water, which is referred to as respiration and transpiration. It is through the process of photosynthesis that plants not only maintain physiological activity, but also take up excessive ozone, which enters the stomata along with CO_2. While CO_2, light energy, and water and nutrients are moving throughout the plant and being processed into sugars, ozone is carried along. As a leaf ages, ozone-induced foliar injury presents itself as dark colored spots (stippling) visible on leaf topsides. Symptoms are discussed in further detail for each bioindicator species subsequently.

Vascular tissue or veins. Leaves have long strips of vascular tissue or veins. In the center of the leaf is a central vein, or midrib. There are a number of smaller veinlets called side veins that extend off the midrib and extend to the edge of the leaf. The roots of the plant absorb water and minerals from the soil; water and minerals travel up the stem into the leaf petiole and end at the leaf midrib vein and then out to the leaf edge through the veinlets. The vein system supplies all parts of the leaf with water and minerals it will need for photosynthesis. Veins and veinlets are the upper end point of the continuous flow of the vascular water and nutrient transport system that originates within the root hairs that extend out into the soil matrix.



All broad leaf plants use the sun's energy to produce their food through leaves specially adapted for photosynthesis. The cross section of the leaf highlights some of the special cells inside a leaf with an overview of the leaf parts and how each part is involved in photosynthesis.

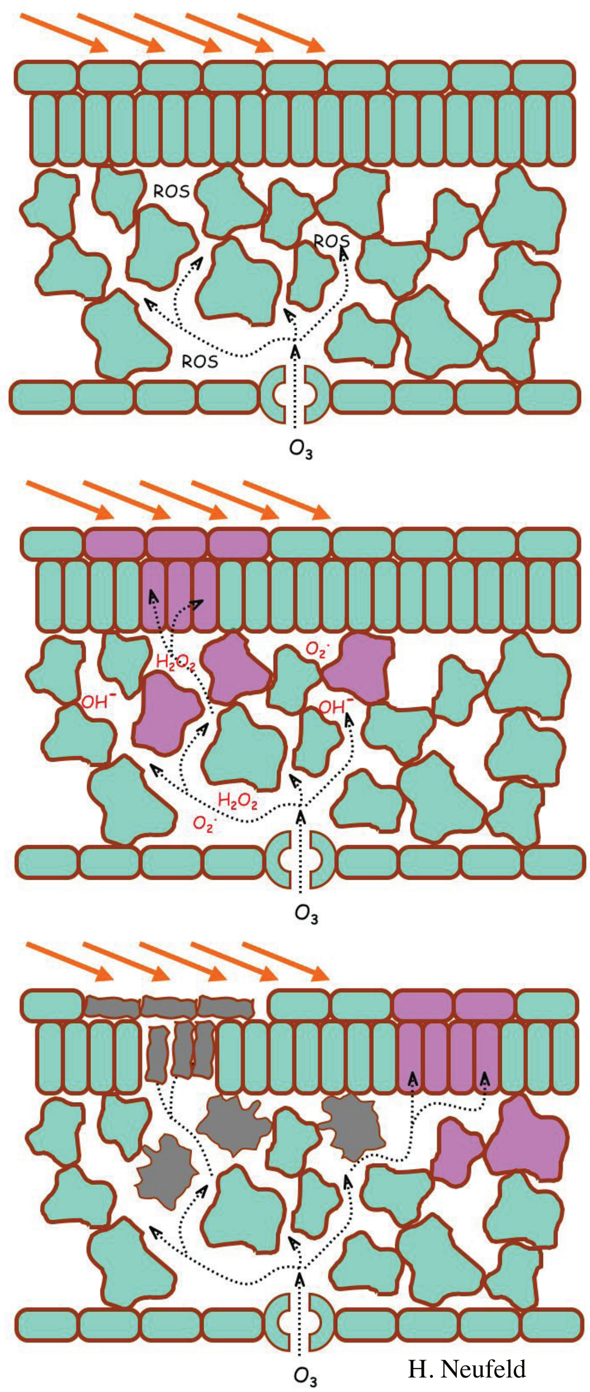
Upper epidermis. The upper layer of the leaf is called the upper epidermis. It is a layer of flat wax-covered cells on the leaf's surface. The cells of the epidermis layer have stiff cell walls, which help to protect the leaf and give it its shape. The waxy surface (cuticle) of the leaf helps to keep moisture inside the leaf from evaporating. Photosynthesis could not take place if a leaf lost all its water.

Palisade mesophyll. The palisade mesophyll layer is located just underneath the upper epidermis. These cells are tightly packed and have great numbers of tiny green chloroplasts. The microscopic chloroplasts contain the chlorophyll that is needed to absorb the sun's energy. These chloroplasts work like solar collectors, using the sun's energy to power photosynthesis. Photosynthesis happens faster and to a much greater extent on bright sunny days because more direct energy comes from the sun. The plant then produces more oxygen and food than it can use. The unused oxygen is given off into the air and the extra food the plant produces is stored as starch.

Spongy mesophyll layer. The spongy mesophyll cell layer lies directly under the palisade cell layer and comprises a variety of loosely organized shapes of spongy cells and air space. Air entering a leaf through its stomata can easily move throughout the large open spaces found in this spongy layer of cells where gas exchange and photosynthesis also take place.

Stomata. Leaves have tiny pores called stomata (singular: stoma) that are used for gas exchange, including water vapor. Each pore is made up of specialized cells called guard cells. Guard cells regulate a stoma's opening through turgor pressure during periods of good water supply. They close the stoma by collapsing during times of dryness and increased temperatures to reduce transpiration and prevent water loss.

The Progression of Ozone-Induced Foliar Injury Through Photosynthesis. All gases entering a leaf follow the same path once inside. Within the air, CO_2 and other gases including ozone (O_3) air pollution enter the leaf. The following three schematics show how light and ozone interact to produce stippling, discrete and very small angular areas of pigmentation that are visible only on the upper leaf surface.



H. Neufeld

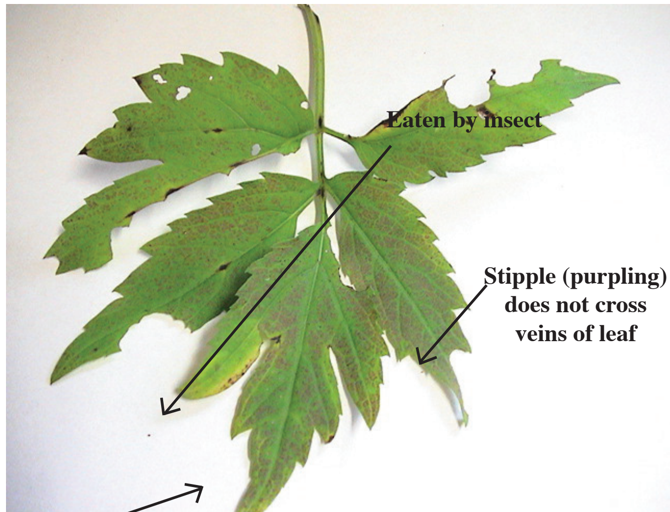
Ozone is a highly reactive molecule and once it enters through the stoma it finds its way through the leaf interior to the cells responsible for photosynthesis, particularly the palisade and spongy mesophyll tissues. The enhanced airspaces in the spongy mesophyll area allow ozone to move freely inside the leaf.

As soon as ozone enters, it most likely reacts with molecules in the cell wall that end up triggering the production of the reactive oxygen species (ROS) molecules, for example hydrogen peroxide, H_2O_2, and hydroxyl radical (OH). Ozone itself is believed to rarely make it far enough into a cell to cause direct chemical toxicity.

The darkened leaf cells are those stimulated to produce anthocyanins, or the pigmentation that then accumulates within the injured cells and causes the dark colored upper surface stippling.

The brownish-gray cells on the upper side of the leaf to the right have died and shrunk, leaving necrotic regions on the leaf. The three illustrations show a progression of injury from initial attack to first stippling, then the spreading of stippling to other cells, and finally necrosis of the first cells attacked.

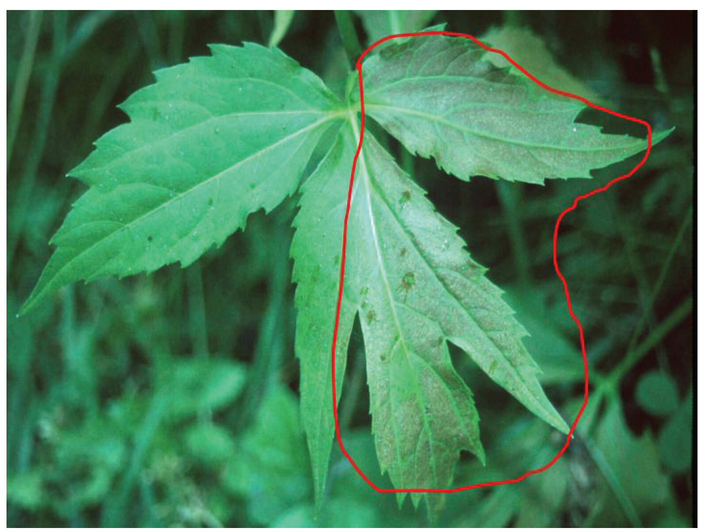
Even though ozone primarily comes through the lower epidermal stomata, it is the upper layer of the leaf that shows ozone-induced stipple. The palisade cells suffer injury first, due to their interaction with sunlight, in reactions not yet fully understood.



Chlorotic symptoms (the yellow) is stress respone in leaf

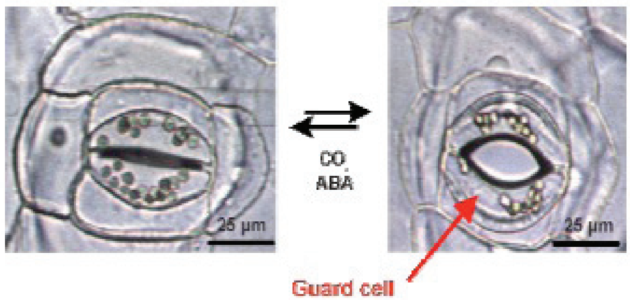
Necrotic region (cells have started to die and shrink)

The uptake of ozone depends entirely on whether the stomata are opened or closed. Stomata open in response to certain environmental stimuli such as light, high humidity, and warm temperatures. At night, stomata tend to close because of the lack of light, therefore ozone uptake is greatly reduced at this time of the day. A decrease in humidity or extremely hot temperatures can cause excessive water loss from the cells surrounding the stomatal pore, and the stomata close.



Note that one side of the cutleaf coneflower leaf has not had any injury (shaded by another leaf) and the other side has very, very light injury (fully exposed to sunlight). The stomata on the left side of the leaf did not open due to shading, unlike the half exposed to sunlight.

A stoma comprises two guard cells and some accessory cells that assist in opening and closing. When the guard cells fill with water, they pull away from each other, and the stomatal pore is opened. When the guard cells lose water, they collapse against one another, closing the pore. If a plant is suffering from drought stress, stoma close, leading to a conservation of water. While this may prevent the plant from drying out, it also lowers photosynthesis because the carbon dioxide cannot get into the leaf. One benefit is that ozone cannot get into the leaf and thus plants under drought stress show fewer ozone-induced symptoms than well-watered plants.



Closed stoma Open stoma

Ozone-Induced Symptoms on Broadleaf Plants. Ozone-induced symptoms typically develop on the foliage of sensitive plant species during the late spring, summer, and early fall following exposures to ozone air pollution. Some degree of expertise is required to recognize ozone-induced foliar symptoms, but with careful observation of the specific symptoms as described herein, accurate diagnosis is possible. Observers must keep in mind that many symptoms of disease and insect activity are also likely to be present on the ozone-sensitive plants and include conditions such as normal senescence (growth and aging), nutritional disorders, other abiotic stressors, biotic pathogens, or insect infestations. For purposes of this program, it is important to recognize and evaluate only the specific symptoms of ozone-induced injury.

Snap bean with ozone-induced symptoms, K. Burkey, USDA-ARS

Cutleaf coneflower with ozone-induced symptoms

The most commonly observed symptom of ozone exposure is the presence of stipple on the upper leaf surface of sensitive plants as seen on the snap bean, cutleaf coneflower, and milkweed leaves pictured. Leaf surface stipple has been described as the classic symptom of ozone-induced injury on broadleaf species.

The upper leaf surface of ozone-sensitive plants may exhibit minute tan, brown, red, purple, or black coloration that appears scattered over the upper leaf surface. Observe the dark stipple on the surface of the milkweed leaf. The coloration of stippling is usually characteristic for a species but can vary with environmental or physiological conditions.

Milkweed with ozone-induced symptoms

Stipple may be restricted to certain areas of the leaf, but the veins and small veinlets are not involved; very small veinlets often border injured areas producing angular appearances of the affected tissues. Very fine or newly developing stipple is best observed with a 10x magnifying glass. As the summer season and ozone-exposures continue, the entire surface of older leaves on sensitive species may exhibit near 100-percent symptoms. Older leaves are located closest to the plant's base and exhibit more stippling due to increased exposure durations over a season. A more general upper surface pigmentation may also occur due to season-long ozone exposures.

Chlorosis, or yellowing due to lack of green chlorophyll pigmentation, of older leaves may also take place as ozone exposures increase throughout the summer season. Caution must be used in interpreting this symptom due to numerous other factors causing yellowing, but when in concert with upper-surface stipple, the yellowing is likely a part of the progression of ozone-induced injury.

Ozone injury is usually expressed as upper leaf surface stippling with the lower leaf surface clear of symptoms; stipple is not present on the veins or veinlets.

Necrosis, or death of leaf tissue, occurs prematurely after injury and damage have become extensive. The tissue turns brown and the wilted, drying leaves either hang from the stem base or drop off the plant. Premature leaf drop may occur as early as middle to late July depending upon species sensitivity, ozone concentrations, and local weather conditions at the site. More specific descriptions of ozone injuries and lists of sensitive plant species under natural conditions and ambient ozone exposures have been published (Krupa et al., 1998; Skelly et al., 1987 & 1998; Innes et al., 2001).

Common Plant Species Known to be Ozone Sensitive. Around the perimeter of the bioindicator gardens, project participants are encouraged to look for symptoms of ozone-induced foliar injury (primarily upper leaf surface stipple) on a variety of other plant species known to be sensitive to ozone air pollution. Many listings are available in the diagnostic guides as cited within this manual. Some caution must be used in late season assessments as many of these species have natural reddening and even purple coloration showing during the early to late autumn season.

Studies of Ozone-Sensitive Native Plants. Many studies and recently published reports in the U.S. have documented ozone-induced foliar injury on ozone sensitive plant species. Long-term investigations of selected plants have been conducted in open-top chambers in the Great Smoky Mountains National Park of Tennessee and North Carolina, the Shenandoah National Park in Virginia, and the Allegheny Mountains of north-central Pennsylvania (Chappelka et. al., 1992; Neufeld et al., 1992; Hildebrand et al. 1996; Skelly et al., 1996). This guide provides information specific to snap bean, milkweed, and cutleaf coneflower because these species fit the criteria for being good bioindicators of ozone-induced injury. Other ozone sensitive agricultural and native cultivars are not covered in this guide.

Not All Plant Injury is Ozone-Induced

Mimicking Symptoms. When assessing the percentage of injury to a leaf, awareness of other causes of leaf injury is important. A critical consideration is that the bioindicator garden should be in an area free of herbicide applications or risk of mechanical or physical injury (i.e., landscape or farm equipment). Insects also cause injury to broadleaf plants, but this injury looks very different from stippling caused by ozone air pollution—symptoms usually occur on both the upper and lower sides of a leaf. Several biotic pathogens including fungi, viruses, and bacteria infect snap bean, milkweed, and cutleaf coneflower leaves. These symptoms usually involve both upper and lower leaf surfaces and are easily distinguished from the typical angular and upper surface stippling caused by ozone exposures.

Leaf hopper injury on snap bean leaf
M. McGrath, Cornell University

The injury on this leaflet of snap bean was caused by a leafhopper, not ozone. The leafhopper is about one-eighth of an inch long and, with its wing structure, is a wedge-shaped insect. Leafhoppers and aphids damage a leaf by piercing it and literally "sucking" nutrients out of vascular tissues. The necrotic (brown) injury on the leaf appears on the **upper** and **lower** sides.

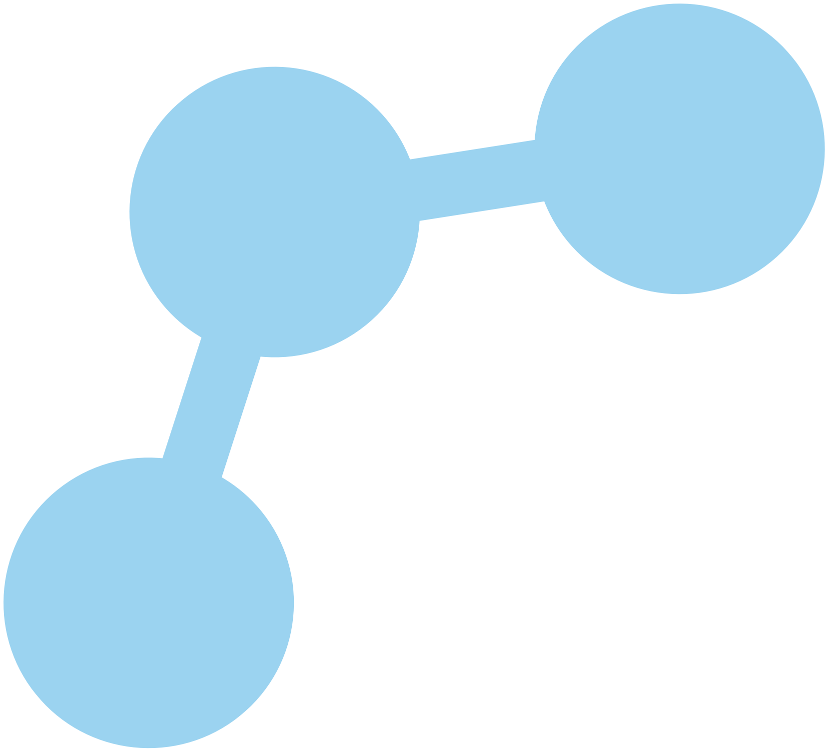
Bean Leaf Beetle
W. Upham,
Kansas State University, PDIS

Other symptoms of leaf injury may be caused by insects simply grazing on leaf surfaces and edges. A 10x magnifying glass greatly enhances the ability to see these very small insects and many other surface symptoms exclusive of the typical stippling caused by ozone.

Milkweed bettle
P. Sloderbeck,
Kansas State University, PDIS

Another type of leaf injury <u>not</u> caused by ozone is powdery mildew. It appears as a dusty white to gray coating over a leaf's surface. There are several types that affect all kinds of broad leaf plants. Powdery mildew appears on the upper and under sides of leaves.

Mimicking symptoms caused by disease or insects are common, and the observers of ozone-induced foliar injury are reminded to carefully review the specific characteristics of ozone-induced symptoms for each plant in the field guide identified as injury that is NOT ozone-induced. If available, another option is to ask for the assistance of your local agricultural or forestry extension agent. Further descriptions of mimicking symptoms are available within the field guides for each bioindicator species.



Section II

The Ozone Bioindicator Garden

As an indicator of plant stress, ozone sensitive plants provide a tool to detect and monitor local changes in air quality and effects on the local environment. More specifically, the ozone bioindicator garden is designed as a tool to detect and monitor ozone stress on sensitive and tolerant plants. The focus of this manual is to develop a bioindicator garden with four plant species known to be easily established and maintained: the snap bean (*Phaseolus vulgaris*), common (*Asclepias syriaca*) and tall milkweed (*Asclepias exaltata*), and the cutleaf coneflower (*Rudbeckia laciniata*).

Planning the Ozone Bioindicator Garden

An open area of at least 10 to 15 m (30 to 50 ft) on either side of a garden is necessary to allow good air movement and normal precipitation without "rain shadows," and of greatest importance, the plants must receive direct sunlight. A reasonably good natural field or cultivated soil is recommended for the site. It is important to be able to water the plants during a dry season or periods of little rain to promote proper conditions for the development of ozone-induced foliar injury. Stomata remain closed and photosynthesis decreases significantly during dry periods, resulting in leaves taking in less air and therefore having less foliar injury during periods of higher ozone exposures. Watering the bioindicator garden will support active photosynthesis and the uptake of ozone in the sensitive plants, and thus more injury may be observed.

The garden's location must be away from areas where pesticides and fertilizers are used. It is important that no pesticides or fertilizers are used in the ozone bioindicator garden. In selecting a site, some compromise may be necessary between an ideal location for scientific observations and the logistical constraints of an area and its surroundings. To ensure the value of data documentation, the nature of a bioindicator garden site and its surroundings should be recorded using the site definition sheet found in the appendix. Also, enclosing the garden with fencing and a gate will prevent unwanted traffic and plant injury due to machinery, animals, or vandalism.

The garden's size and shape depends upon available space, number of observers expected to visit the site, and number of plants used. It is recommended that the initial garden include four plants from each species and cultivars within each species planted

35 to 40 cm (15 to 18 in.) apart. Space to walk through and around plants is critical to maintaining a plant's natural health and preventing injury during observations. This keeps the garden manageable and allows growing room along with expansion to include more plants as the garden matures.

If a gardening area is not available, nursery pots may be used for snap beans, using the planting directions identified in the guide. A large light colored pot about 60 cm (2 ft) in diameter and filled with natural soil may be used for one coneflower or milkweed plant.

Designing a Small Garden. If land for a garden is limited, it is recommended that the snap beans be grown in nursery pots and cutleaf coneflowers and milkweed be planted in natural soil. Remember, watering is important; there needs to be a source nearby. The smaller garden may be 1 m (3.5 ft) by 2 m (6 ft) with three cutleaf coneflower plants on one side and three common or tall milkweed plants on the opposite side.

Allow about 35 to 40 cm (15 to 20 in.) between each like plant that is planted along the long side of the garden. The cutleaf coneflower will eventually develop a "rosette" at its base from which a new plant will grow the following year. The rosette grows to about 30 cm (12 in.) in diameter. The mature milkweed plant's leaf spread eventually reaches a similar diameter.

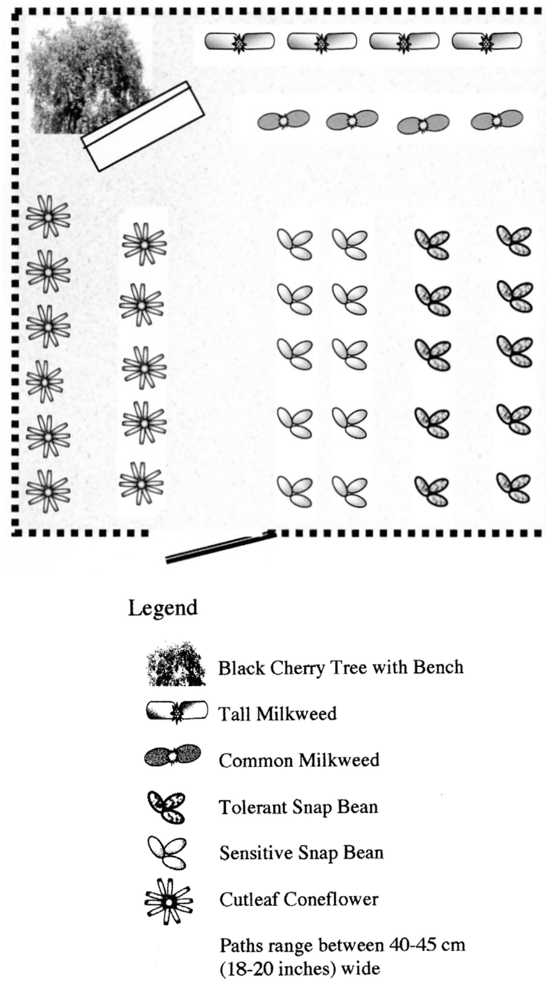
Protect the ozone bioindicator garden with some type of fencing that will allow air to circulate freely but prevent small animals from entering and eating the plants. Cover each path with landscape cloth, which is permeable to rainfall, and then mulch to minimize weeding. Landscape cloth placement and mulch will identify walking areas for observing plants.

If a larger area of land is available, the design of the bioindicator garden can contain ozone sensitive bioindicator species and perhaps a few other ozone sensitive plants to create an aesthetic layout.

- A larger garden design of 3.9 m^2 (10 ft^2) will provide a more flexible planting layout.
- Allow 35 to 40 cm (15 to 18 in.) between like plants within each row.
- Enclose the garden with a fence (preferably chain link or chicken wire) with the bottom edge buried 5 to 6 in. into the ground to protect it from unwanted animals. Provide a gate for a designated entrance.

- Allow 55 to 60 cm (20 to 24 in.) for paths between rows of plants and cover the walking paths with landscape cloth and mulch.

Plant spread is controlled by digging up and removing at each season's end any unwanted plants that may have spread by means of underground rhizomes (roots) or by new plants emerging from seeds the following spring. Allowing a second plant to grow next to each original plant affords the opportunity to double each species for data gathering.



Organizing Plants for Monitoring

Arranging the ozone sensitive plants for easy access and movement around them is important to successfully observe plants for ozone-induced injury. Observers need to be able to get close to each plant without walking on or damaging others. More detailed information for planting each cultivar may be found in the designated sections of the implementation guide.

The observer needs to design the site map showing the location of paths and plants, any buildings or other large plants nearby, and compass points indicating north, south, east and west (N, E, S, W). Mark north in the garden plot with a stake to maintain a sense of direction, a necessary designation once tagging leaves begins.

Setting up Plant Identification (ID). As each plant begins to grow, gently drive a 1.5 m (about 4 ft) stake about 30 cm (12 in.) into the ground next to the plant. Use a permanent fine-tip black marker to write the plant's ID code on the upper part of the stake. Loosely place a tag with a sturdy wire or plastic tie at the base of the plant's stock, which will enable plant identification in the event the stake gets removed.

Tagging the leaves. The next important task is coding each plant and labeling each of the leaves selected for gathering data. Establishing plant IDs and tagging leaves are the most time consuming tasks in organizing plants for observations of individual leaves and the entire plant. Specific procedures are imperative to data-gathering accuracy, particularly if more than one person is involved in making observations. The following steps are for initially preparing four plants of each species for observation.

Snap Bean. There are two conventions for identifying snap bean leaves, but the overall ID process and leaf labeling for each plant remains the same. Each plant must be labeled as tolerant or sensitive and numbered so the observer will record the data specific to each plant. The same labeling procedure is followed for planting in tilled soil and in nursery pots. The process for bean plant identification and leaf labeling is:

- Ozone tolerant (T) bean (B) plants in the field or in pots must have an ID at the base indicating it is ozone tolerant, using these codes: TB1 for the first plant, TB2 for the second, TB3 for the third, and TB4 for the fourth.
- Ozone sensitive (S) bean (B) plants in the field or in pots must have an ID at the base indicating it is a sensitive plant using these codes: SB1 for the first plant, SB2 for the second, SB3 for the third, and SB4 for the fourth.

Leaves are labeled from the base of the plant to the top as they begin to emerge. New trifoliate leaves emerge along the stem as it elongates from the terminal apex (upper tip of the stem), therefore the oldest single foliate leaves are at the bottom of the plant. When assessing a trifoliate leaf for ozone-induced injury, the observer must look at all three leaflets. The trifoliate leaf is evaluated for injury as one leaf. The trifoliate leaves grow randomly around the stock of the plant, so it is important to tag each leaf as it appears. This enables the observer to know which leaves are older and which are newer.

It would be advantageous to write out the small tags, using a fine point permanent marker, before the leaves begin emerging and place leaf tags in a zip lock bag labeled with the plant's ID number (TB1) and leaf number (TB1-1 or SB1-1 series, etc.). For example, the leaf tags for TB1-1 would have this plant's ID on the tag followed by -1 indicating it was the first leaf to emerge on the stem of that plant. Leaf tags for this one plant would read: TB1-1 for leaf one; TB1-2 for leaf two; TB1-3 for leaf three; and this pattern is repeated until the first 10 trifoliate leaves have been tagged for this specific plant. The same steps would be followed for the other tolerant and sensitive bean plants.

Cutleaf Coneflower. Each plant is tagged at the base with a number using CF for coneflower. Each of the four coneflower plants needs to have an ID attached to its base and also written on a 1.5-m (59 in.) wooden stake at the base of the individual plant.

Use this convention to ID each plant and label leaves:

- The ID for each plant: CF1 for the first plant, CF2 for the second, CF3 for the third, and CF4 for the fourth.

Starting from the **bottom of the stem**, the first leaf is tagged as #1, then move in a clockwise fashion up the stem. Label the next leaf in sequence #2 and continue numbering up to the tenth leaf.

The coneflower leaves on the first plant coded CF1 would be tagged CF1-1, CF1-2, CF1-3, on up to leaf number 10. The second, third and fourth plants follow the same pattern using their specific plant ID: CF2, CF3, and CF4. Use a black fine-tip permanent marker and *gently* write the leaf code on the underside of each leaf.

Only the 10 bottom leaves are numbered and if, after labeling, a leaf falls off near the bottom of the plant, it is still counted as one of the original 10 leaves used for data gathering. How to include the dropped leaf in the data gathering is discussed later in the coneflower section.

At the end of the season, the stakes will identify each plant from which observers have gathered data and will be a marker to identify the new plants as they emerge the following spring.

Common and Tall Milkweed. The four milkweed plants need to be identified on the garden site map as either common or tall milkweed. The following process for each plant's ID and leaf labeling is recommended to ensure consistency and accuracy in data gathering:

Each of the four milkweed plants need to have an ID attached to its base and also written on a 1.5-m (59 in.) wooden stake at the base of the individual plant.

- The ID for each milkweed species begins with C for common and T for tall followed by MW for milkweed. The following sequence would be used to ID four common milkweed plants: CMW1, CMW2, CMW3, and CMW4.

The leaves on the milkweed plant are opposite one another. **Labeling of leaves starts from the bottom of the plant and progresses upward toward the top**. While facing north, as leaves emerge during the growing season, the first leaf pair is labeled leaf 1A and 1B. As the next leaf pair emerges, the leaves are labeled 2A and 2B and so on until the first eight pairs of leaves have been produced. These leaf tags should be prepared and ready to put on the stem of each leaf as the new leaf pairs emerge. Repeat this process for the three other milkweed plants.

Be sure to put the plant ID, for example CMW1, on the top of the data sheet and collect data specific to that plant. Careful labeling of plants will enable several people to accurately identify each plant for recording data. Each plant is tagged at the base with a number using CM or TM for common or tall milkweed respectively.

Training to Observe and Assess Plant Injury

This segment introduces how to observe and assess ozone-induced foliar injury on the leaves of a plant. Initially, assessing foliar injury begins with using a 10x magnifying glass to closely examine the leaves until the first symptoms of ozone-induced foliar injury appear. Initial observations for ozone-induced foliar injury should be started around May or June, depending on the ozone bioindicator garden's geographic location and the likelihood of higher local ozone pollution exposures. Plants should be assessed for symptoms only on bright sunny days and with the sunlight over the observer's shoulder. Observations for symptoms should be continued at 2-3 day intervals, until the first symptoms of typical ozone-induced injury appear. Continue assessing the plants once a week, on the same day of the week or as close as may be possible, providing there are sunny conditions. Assessments may be made until the first frost in the fall of the year.

Measurement Supplies. It is recommended that the observer have a tote bag for carrying the necessary supplies to and from the field. The assessor will need to have:

- A 10x magnifying glass for symptom observation
- A meter stick and small flat board to support the meter stick on the soil surface when recording each plant's height
- Data sheets for each plant
- A clipboard and a pencil
- A fine tip permanent black marker for identifying leaves
- Paper tags with string to tie to each leaf petiole
- A plastic tie for labeling the base of each plant

An ozone bioindicator garden provides the opportunity to measure the amount of ozone-induced injury to plants and to observe the impact of ozone air pollution on ozone-sensitive plant species over time. Accuracy in assessing foliar injury requires practice and an observer can practice and refine skills at http://ozonegarden.larc.nasa.gov/.

- Practice estimating foliar injury on snap bean, milkweed, or coneflower. A series of photographs of ozone-induced foliar injury on a specified plant will appear and offer a choice of estimated ranges of injury. Once a range of injury is selected, the Web site will identify if the selected estimate of injury is correct or incorrect.
- Observers may want to practice estimating foliar injury on the Web site using the field guide and data recording sheets.
- Before each and every field observation period, the observer should repeat this exercise at least three times or until a score with 80-percent accuracy is reached on a session of 10 leaves.

Implementation Ideas

The ozone bioindicator garden promotes environmental awareness for all age groups, through formal and informal educational settings, and provides an opportunity to investigate local air quality questions through observations and data gathering. Investigating visible symptoms on sensitive plants in the ozone bioindicator garden introduces ozone air pollution's effects on Earth's natural systems and makes connections for the participants between daily activities and the environment. The following suggestions are a few ideas for implementing and adapting the bioindicator garden in different settings.

Science and Education Outreach Centers. Informal educational groups interested in investigating how air quality impacts their local environment can use the ozone bioindicator garden as a tool to answer local air pollution questions.

- Design and plant a garden with ozone sensitive plants to investigate the presence of ozone air pollution and its impact on local vegetation.
- Promote air quality awareness through workshops involving community members in observing and gathering data from plants showing ozone-induced foliar injury.
- Promote a youth environmental day camp focusing on air quality. An activity might include using local automated hourly ozone data and correlate it to the percentage of ozone-induced plant injury.
- Create a video about the garden over time to demonstrate the changes to the plants caused by ozone air pollution.
- Sponsor an ozone air pollution awareness day for the community as part of Earth Day with exhibits, posters, and information on the ozone bioindicator garden, and offer opportunities for hands on activities in the garden.
- Train participants interested in setting up their own bioindicator garden and provide follow-up support.
- Construct a display of periods of episodic ozone air pollution and pictures of ozone-induced foliar injury that may occur following an episode of air stagnation.
- Create an environmental calendar about the ozone bioindicator garden covering its implementation, plant growth patterns, observations and data gathering, photos of plants showing symptoms of ozone-induced foliar injury, and ozone air pollution levels.

Scouts (girls and boys), 4-H Clubs, and Other Youth Groups. Organize an environmental awareness program for Earth Science by implementing an ozone bioindicator garden with opportunities to: design the garden layout; plant, maintain and manage the garden; observe plant responses to ozone air pollution; and assess the crop size and yield for the two bean varieties.

Participants may be trained to measure levels of ozone air pollution using the scientific protocol and instruments designed by the science-educator team at NASA's Langley Research Center for the Global Learning and Observations to Benefit the Environment (GLOBE) program. Information can be found at **www.globe.gov** in the atmosphere chapter of the.teacher's guide.

- Participants earn merit badges while assisting in managing and collecting data in the bioindicator garden.
- Train interested youth to create their own mini-garden consisting of other species of ozone sensitive plants (using the lists provided within the reference section) to observe the impact of ozone air pollution.
- Sponsor a career day for youth to meet with environmental scientists (plant pathologists, atmospheric scientists) and experience hands-on activities in the bioindicator garden.
- Organize a group visit to a national park (if one is near) and let the youth group tour the park to learn how they monitor air quality and possibly use plants as bioindicators.

Classroom Setting. Planting an ozone bioindicator garden promotes environmental awareness ranging from basic germination of a seed and the impact of sun and water on plant growth to advanced understandings of how human activity impacts air quality and vegetation. The following implementation ideas for the classroom are only a sample of the potential uses of an ozone bioindicator garden from which to launch programs meeting local education needs.

- Read NASA's picture book, The Air We Breathe, to introduce the atmosphere and its importance to Earth's Systems.
- Use the Ozone Bioindicator Garden Implementation Guide as a tool to develop an environmental awareness program and integrate it within core curricula.
- Plant an ozone bioindicator garden utilizing different age level students to perform age appropriate activities. For example: younger students may be

involved in planting, monitoring, and recording plant growth to share with older students who monitor plants for ozone-induced foliar injury. Young students may report or present their scientific findings to the older students, and more technical information gathered by older students may be presented to parents or community groups.

- Use the data gathered in the bioindicator garden and local hourly ozone air pollution levels to make connections between daily activities and their impact on the environment.
- Involve parents in the designing, planting, and managing of the bioindicator garden to develop a core team to coordinate summer monitoring with students, when ozone air pollution levels are highest.
- Coordinate a summer environmental camp supported by parent volunteers to have consistent and accurate data and develop public awareness of the impact of degraded air quality on vegetation.
- Near the end of the ozone air pollution season, collect leaves with various ozone-induced injury levels, press and dry them, and laminate them for future use for workshops, training, or presentations. You can also scan the leaves to create computer image files to present electronically.
- During the early part of the growing season, take close-up digital photos of healthy plants and document the progression of ozone-induced stipple (purpling) as air quality changes. Create a photo exhibit along with summer-long ozone exposure patterns to explain the impact of air quality on vegetation.
- Write articles about the ozone bioindicator garden summarizing experiences and understandings.
- Develop teams to monitor a specific plant's response to ozone air pollution and have each team present to compare findings with other teams. Each team's digital photos and data may be recorded in an electronic journal that could be accessible between local schools and accessible to other schools, clubs, community organizations, or scientists.
- Access environmental information specific to your local area at www.epa.gov/enviro/wme/ enter their zip code, town and state and get a "Window to My Environment." When the window is created, zooming out on the window will reveal regulated sites of water discharge, air emissions, toxic release and hazardous waste areas, and multi-activities. The map can be redrawn to identify locations of air monitors.

- Students can explore how human activities and meteorological conditions influence ozone air pollution episodes produced through a simulation environment at www.smogcity2.org.
- Sponsor an Environmental Awareness Day with students as guides in the garden modeling the scientific process they used to gather data on the presence of ozone air pollution, and write songs and music with an air quality theme for presentations. Involve the students in planning, organizing, and implementing the awareness day.
- Submit information about the bioindicator garden and activities implemented with NASA's Langley Research Center at http://ozonegarden.larc.nasa.gov.
- Provide participants in the project with certificates of recognition that may be downloaded at http://ozonegarden.larc.nasa.gov.

NASA Langley Ozone Bioindicator Garden Web Site

Submitting and Retrieving Plant Data. NASA Langley's ozone bioindicator garden Web site is under development, and the ability to submit data is planned for the future. Plans for membership status allowing one to train, submit, and retrieve data will add important interactive features for use by observers of ozone-induced foliar injury.

Until the formal database for submitting and retrieving foliar injury data is activated, participants can go to http://ozonegarden.larc.nasa.gov and click on the "data' link. A sample spreadsheet data page, with directions on how to enter data and create a graph, is available. Template spreadsheets to provide training for data analysis for the snap bean, cutleaf coneflower, and milkweed plants are provided.

Implementation Materials on Web Site. The ozone bioindicator garden Web site will provide implementation materials and interactive activities. Some highlights of the Web site:

- "How to" video clips on planting the bioindicator garden.
- PowerPoint slideshow of what is and what is not ozone-induced foliar injury.
- Electronic version of the *Ozone Bioindicator Garden Implementation Guide* and data sheets.
- Fact sheets about ozone air pollution.

- Additional pictures of ozone-induced foliar injury, sorted by species and percentage of ozone-induced foliar injury, which will enable the observer to compare a range of injured leaves to his or her sample, and estimate the percentage of leaf injury.
- An interactive game is under development using pictures of leaves from each specific plant showing ozone-induced foliar injury to practice estimating the percent of injury.
- An opportunity for participants to submit a site map and photos of their garden sites.

Sources of Ozone Data

Ozone air pollution data in previous studies has been correlated with the percentage of ozone-induced foliar injury to sensitive plants. Local, state, county, and EPA ozone data may be posted daily on their respective air quality monitoring Web sites. However, if hourly daily averages are needed, contact your local air quality agency to request hourly raw data summaries for the period to be studied. Additional information about surface ozone and ozone data may be found at http://airnow.gov. AIRNOW provides a daily map of local and national air quality conditions. Enter a specific state, click on (go) to get a list of ozone monitoring sites and local information. A copy of the Air Quality Index, A Guide to Air Quality and Your Health explaining air quality codes may be obtained at airnow.gov/ikndex.cfm/action=aqibroch.agi.

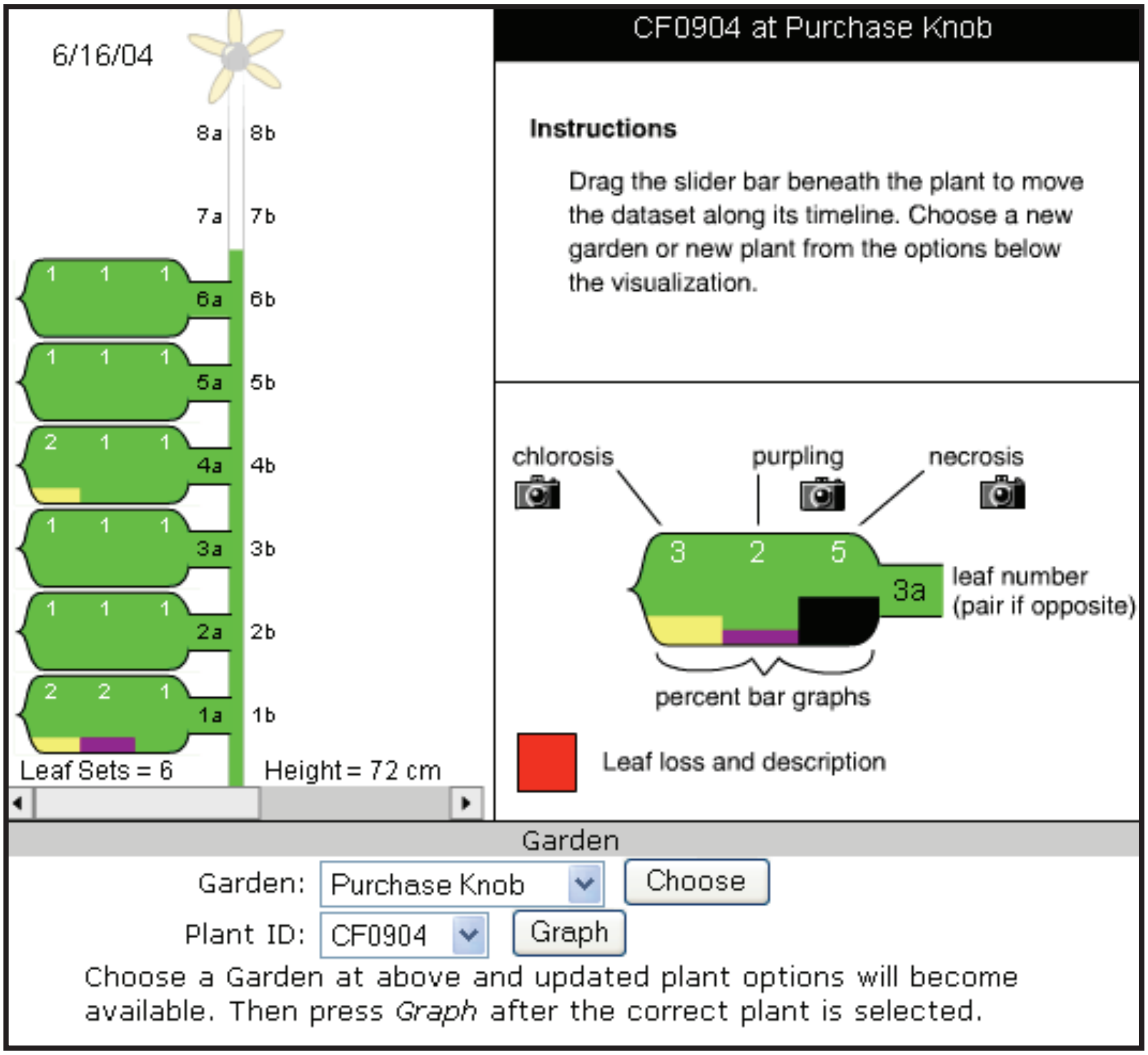
"Window to My Environment" is a powerful tool provided by EPA in partnership with federal, state, local government, and other organizations. This Web-based tool provides federal, state, and local information about environmental conditions and features in any area you select. After choosing a location you can select to search an interactive map for regulated facilities, monitoring sites, population density, perspective topographic views and much more. If you want local geographic statistics go to www.epa.gov/enviro/wme/, enter your zip code and state to create your "window," then select the "your environment" tab.

The GLOBE program offers an international student database that includes a variety of atmospheric measurements. Participants in formal and informal educational settings have been trained to take surface ozone measurements with a hand-held optical scanner to measure local ozone air pollution levels in parts per billion (ppb) and to gather other relevant environmental data: cloud cover and type, relative humidity, wind direction, and current temperature. These data are daily 1-hour samples taken the same time each day within the hour of solar noon. The surface ozone and meteorological data are submitted to the GLOBE Student Data Server www.globe.gov at Colorado State University, Fort Collins, Colorado. Surface ozone data can be retrieved in raw form or in graphic visualizations enabling citizen scientists to analyze their own data for patterns or compare their findings with other sites collecting ozone data.

An opportunity to review ozone-induced foliar injury to plants can be found on the Hands on the Land Web site. The data may be viewed, graphed, or animated over time. Data from two to four gardens may be compared. Go to the Web site at www.handsontheland.org/monitoring/checkup.cfm. Under "environmental monitoring, click on "ozone biomonitoring" to access the page for viewing data.

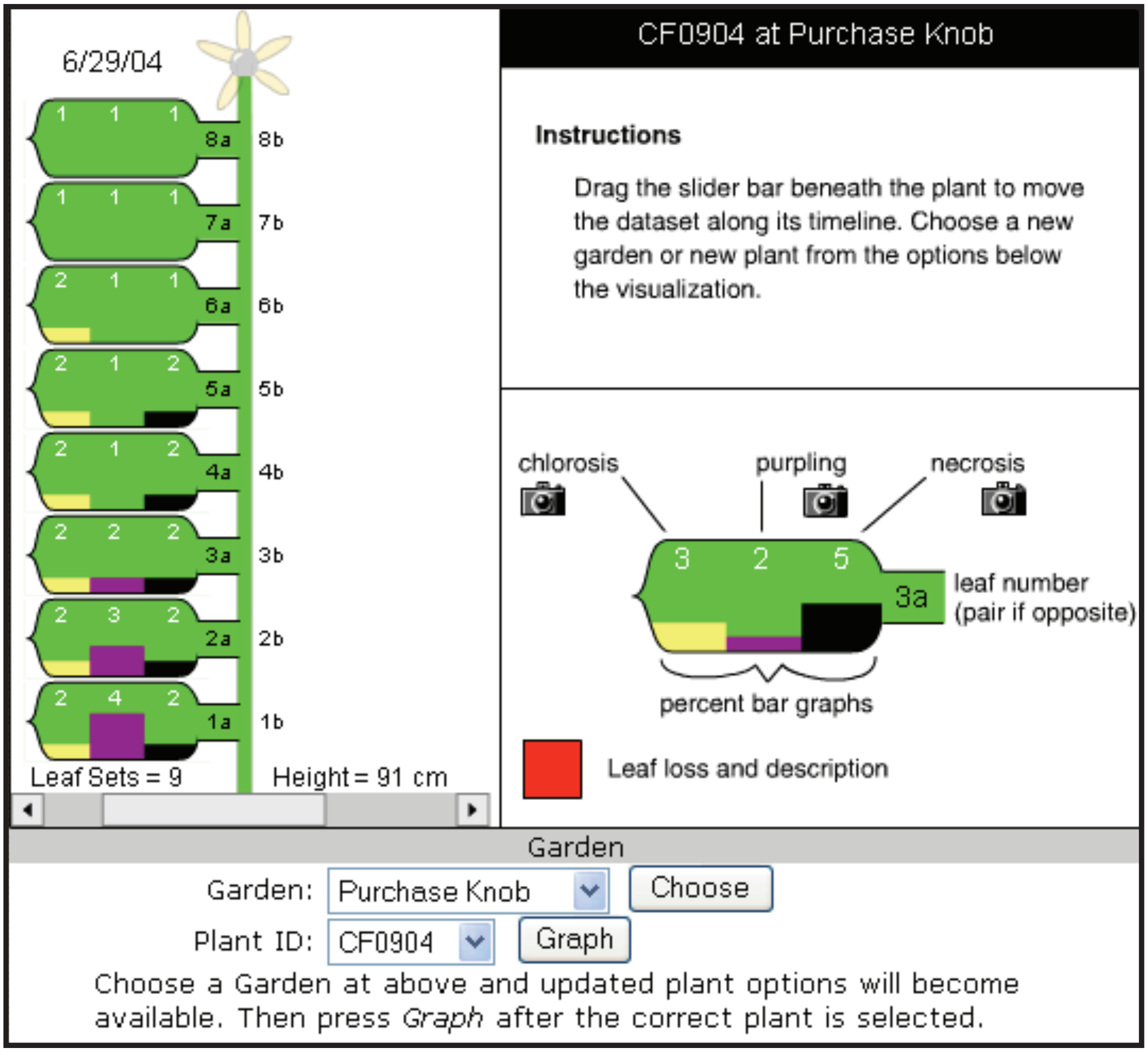
1. **Reviewing data for just one garden**. Select the garden and plant species using the drop down lists. For example, if Purchase Knob is the site and coneflower is the plant, several years of data will be identified.
2. **Comparing Data for Multiple Gardens**. To compare a selected garden with up to four others, click "graph" in the "compare up to four datasets from any garden" in the Reports, Graphs and Maps section.
    a. First select the gardens to compare using the drop down lists that appear after clicking the "graph" button. Select one or up to four sites.
    b. After selecting garden locations, click the "choose garden" button.
    c. Next, choose a plant to compare. In the given example, leaf #4 was selected from one cutleaf coneflower plant at each of these 2004 garden locations Purchase Knob, Cradle of Forestry, and Tuscola. Purpling was the characteristic to be compared and illustrated in a graph format.
3. **Animation of Foliar Injury Over Time**. To see an illustration of ozone-induced symptom progression over the entire ozone-exposure season, choose a garden and click "graph" in the animation section of Reports, Graphs and Maps.
    a. Choose a plant to animate through the growing season and then click "graph." We randomly chose CF0904 from the Purchase Knob garden for the example.
    b. To see the change, slowly drag the slider bar under the graphic of the plant. The dates change and color scales show chlorosis, purpling, necrosis, and leaf drop, reflecting the data collected on each given date.

The following screen shots illustrate that the ozone symptomatic plant showed dramatic changes in its response to ozone air pollution through the growing season.



Screen shot from 6/16

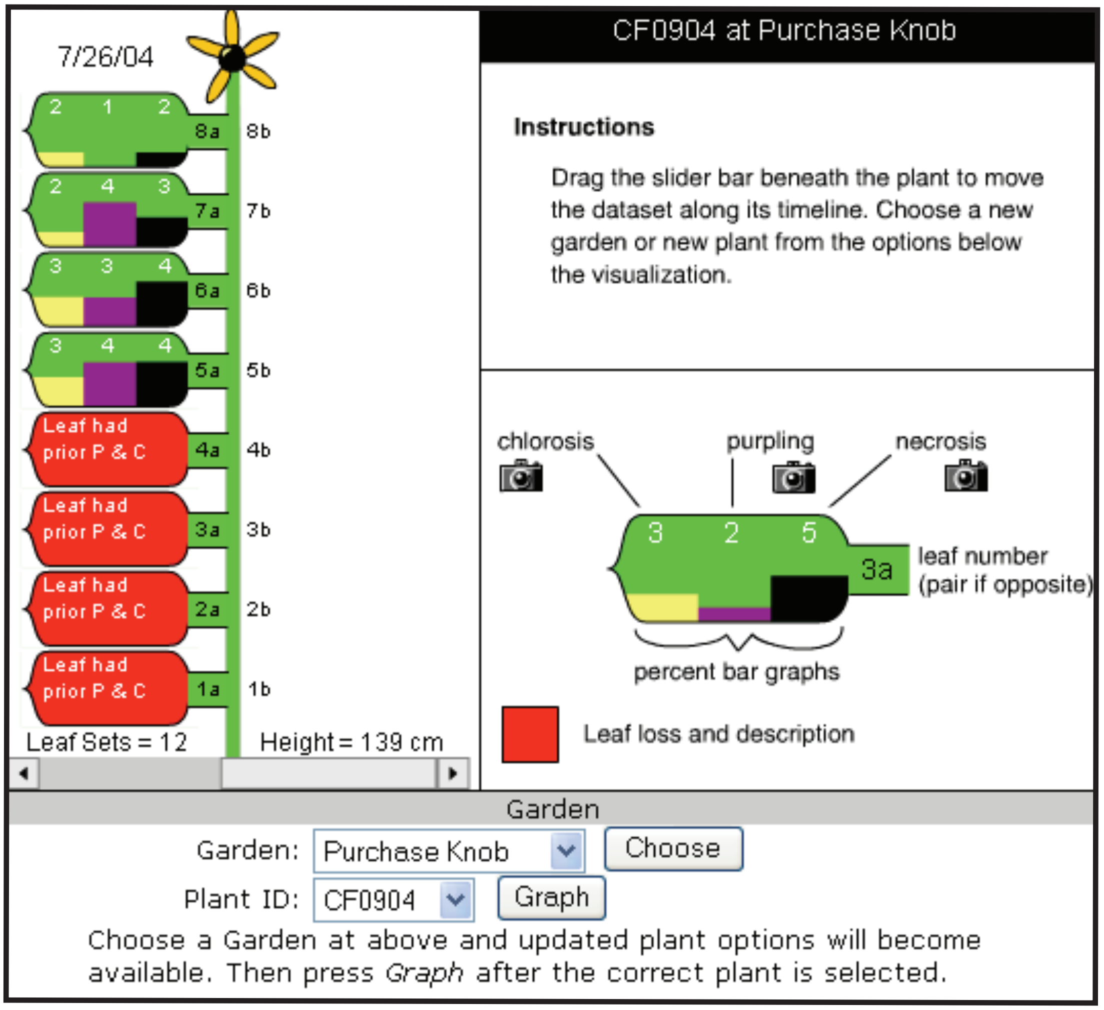
On June 16, 2004, the first data collection date on leaf 1, the plant showed a rating of Class 2 (a percent of ozone-induced injury of 1 to 6 percent for both chlorosis and stippling). Leaf #4 showed a rating of Class 2 (1 to 6 percent) for chlorosis.



Screen shot from 6/29

By dragging the slider bar to the third data collection date of June 29, notice an increase for leaf #1 to a rating of Class 4 for stippling symptoms (26 to 50 percent) and a rating of Class 2 for both chlorosis and necrosis. Both leaf #2 and #3 show purpling symptoms; necrosis is apparent on leaf #5.

By the last data entry on July 26, 2004, the plant had lost the lowest four leaves, and stippling was visible up to leaf #7 with a rating of Class 4 (26 to 50 percent ozone-induced injury).



Screen shot from 7/26

These screen shot examples are from Hands on the Land Web site, courtesy of Susan Sachs, Education Coordinator of the Appalachian Highlands Science Learning Center, Great Smoky Mountains National Park.

Looking at Data

Are the data reasonable? Average plant injury from ozone air pollution can range from 0 to near 100 percent, depending upon the time of year and the accumulative ozone exposures occurring throughout the spring and summer seasons. Research shows that different amounts of ozone-induced foliar injury occur among plants of the same species because of differences in soil, amount of water, ozone exposures, and available sunlight on leaf surfaces and species genetics. However, there are some correlations that usually apply.

1. Sunlight drives the amount of air taken in for photosynthesis. The more active a plant is on days with elevated ozone air pollution, the more likely ozone sensitive plants will show ozone-induced injury.
2. Increased ozone concentrations over time produce increased plant injury. Studies in different regions show that different topographic elevations and amounts of sunlight also influence the incidence and severity of plant injury.
3. Shaded plants or even shading of individual leaves or portions of leaves will show less injury than those plants and leaves located in full sunlight with the lower, older leaves showing most of the injury due to season-long exposures.

Weekly measurements of the percentage of plant injury should provide a general increase in the amount of ozone-induced injury over time. Particular note should be taken of weather conditions (temperature, days with intense sunlight, cloud cover and type, wind direction, and humidity). These factors influence the concentrations of surface ozone present. Were there several consecutive days when the ozone exposure was high? Were there any dramatic changes recorded in the amounts of injury to the plants? On a longer time scale, how did the ozone-induced plant injury vary each month? What can be learned about the effects of ozone air pollution from the observations made of the increasing incidence and severity of ozone-induced foliar injury to plants?

Patterns Observed. One method of collecting data is to estimate foliar injury once a week on the same day of each week. One sample of a complete weekly data set for the crown beard was selected by Susan Sachs to demonstrate how data may be used to observe patterns. The data taken for one season at Purchase Knob Science Center, Great Smoky Mountains National Park, are recorded and represented below in spreadsheets and graphic forms using the Hands on the Land plant coding system. The discussion of the data uses the following definitions and percentage of ozone-induced foliar injury ratings to explain the results of the analysis.

Garden Site: Purchase Knob

Plant ID: CB0104 (crown beard data courtesy of Susan Sachs)

Definitions: Chlorosis = yellowing of areas of the leaf due to ozone-induced injury

Stippling = small dark purple or dark colored speckles appearing on the upper side of the leaf from ozone-induced injury

Necrosis = death of leaf tissue causing it to turn brown

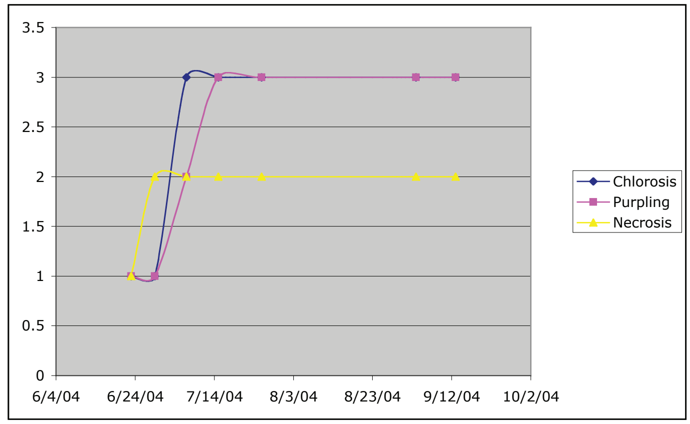
Percent of Foliar Injury on the Upper Side of the Leaf:

Class 1 = 0%	No ozone-induced injury
Class 2 = 1%-6%,	Light ozone-induced foliar injury and beginning symptoms of injury
Class 3 = 7%-25%	Moderate ozone-induced injury
Class 4 = 26%-50%	Moderately severe ozone-induced injury
Class 5 = 51%-75%	Severe ozone-induced injury
Class 6 = 76%-100%	Extremely severe ozone-induced foliar injury usually results in the death of the leaf

The average plant injury from ozone air pollution can range from 0 to 100 percent depending upon the time of year and the accumulative ozone exposures that have occurred throughout the spring and summer seasons. Standing at the plant, facing north, the A leaves to the right of the stem are labeled 1A and 2A, and to the left of the stem, 1B and 2B. Facing north, the right side of the plant is referred to as the east side of the plant, and the left side of the plant, the west side. Data gathering begins from the plant's bottom leaves and then progresses to the top because the bottom leaves are older and exposed to ozone air pollution longer. **NOTE:** Hands on the Land labels the right (east) side of the plant A and the left side (west) B.

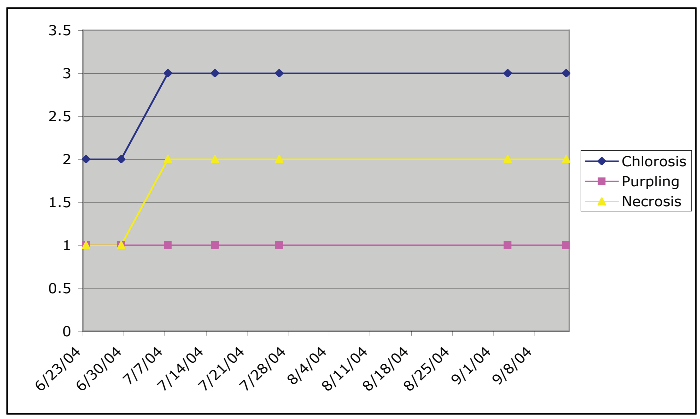
Leaf 1A and 1B are the two bottom leaves on the plant. Review of the data expressed below for the first two leaves during the first two weeks (June 23 and June 29) reveals no ozone-induced foliar injury to either leaf during June. A rating of Class 1 indicates the two leaves to be healthy and free of stipple. However, leaf #1A showed beginning necrosis symptoms near the end of June with moderate chlorosis (Class 3) beginning the first of July and the percentage of necrosis remaining constant during the period of observation.

	LEAF 1 A		
	Chlorosis	Purple Stipple	Necrosis
6/23/04	1	1	1
6/29/04	1	1	2
7/7/04	3	2	2
7/15/04	3	3	2
7/26/04	3	3	2
9/3/04	3	3	2
9/13/04	3	3	2



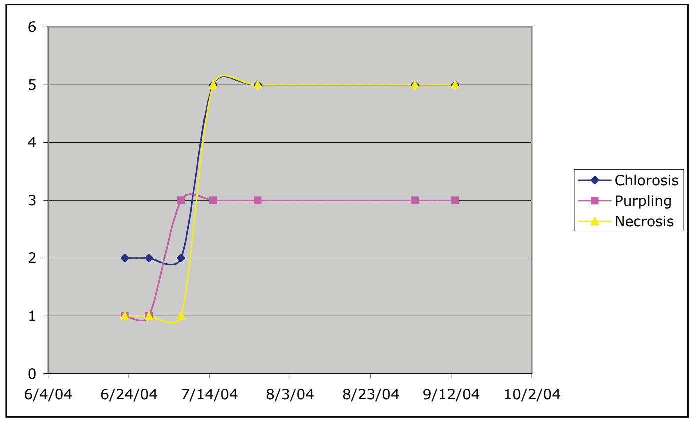
Leaf #1B on the west side of the stem continued to reveal no ozone-induced injury (Class 1) through September, but did show moderate (Class 3) chlorosis and beginning (Class 2) necrosis.

	LEAF 1 B		
	Chlorosis	Purple Stipple	Necrosis
6/23/04	2	1	1
6/29/04	2	1	1
7/7/04	3	1	2
7/15/04	3	1	2
7/26/04	3	1	2
9/3/04	3	1	2
9/13/04	3	1	2



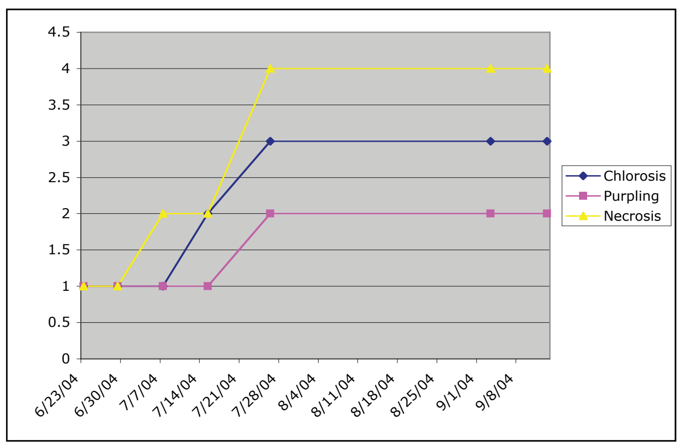
During June the data below shows that leaf #2A and #2B are exhibiting a rating of 1 (no ozone-induced foliar injury) but by July, leaf #2A showed moderate ozone-induced injury (stipple) and leaf #2B remained free of stipple. The last measurements taken July 26 shows leaf #2A's stipple remaining at 7 to 25 percent and not increasing through August, but the leaf shows ozone-induced stress through increased chlorosis and early necrosis (browning), and the beginning of senescence (death).

LEAF 2A	Chlorosis	Purple Stipple	Necrosis
6/23/04	2	1	1
6/29/04	2	1	1
7/7/04	2	3	1
7/15/04	5	3	5
7/26/04	5	3	5
9/3/04	5	3	5
9/13/04	5	3	5

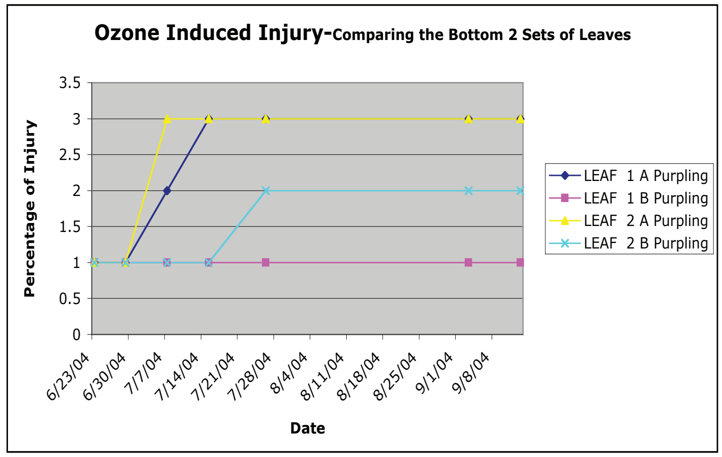


In contrast, the data below show the second leaf on the west side (left side) of the plant does not show an increase in stipple during July, but does show beginning chlorosis (yellowing) symptoms reflecting plant stress. Although light ozone-induced stippling of 1 to 5 percent (Class 2) began to show by the last week in July, the leaf showed moderately severe injury of 26 to 50 percent (Class 4) necrosis (browning) and the beginning of senescence. Leaf #2A showed a high amount of necrosis the second week of July. The leaves on the east side of the stem showed more ozone-induced foliar injury and earlier senescence than leaves on the west side of the stem.

LEAF 2 B	Chlorosis	Purple Stippling	Necrosis
6/23/04	1	1	1
6/29/04	1	1	1
7/7/04	1	1	2
7/15/04	2	1	2
7/26/04	3	2	4
9/3/04	3	2	4
9/13/04	3	2	4



	LEAF 1A Purple Stippling	LEAF 1 B Purple Stippling	LEAF 2 A Purple Stippling	LEAF 2 B Purple Stippling
6/23/04	1	1	1	1
6/29/04	1	1	1	1
7/7/04	2	1	3	1
7/15/04	3	1	3	1
7/26/04	3	1	3	2
9/3/04	3	1	3	2
9/13/04	3	1	3	2



The summary of ozone-induced stippling indicates that the bottom leaf (1A) on the east side of the plant experienced earlier ozone-induced foliar injury than the leaves on the west side of the plant's stem. This pattern is repeated for the second leaf on the east side of the plant's stem (2A) with the leaf on the west side (2B) continuing to show less ozone-induced foliar injury.

Additional Ideas for Data Analysis.

- Select like plants to compare results of ozone air pollution on leaves and see if the patterns observed on the leaves east and west of the main stem in this study are comparable.
- Compare ozone episodes and possible increases in ozone-induced symptoms.
- Compare differences in symptom severity between MW and CF at each site.
- By comparing your sites data and results from other sites, determine how elevations of the sites affect the timing and severity of ozone-induced symptoms.
- Identify the differences in flower and seed production within the tolerant and sensitive bean varieties.
- Determine if there are differences in Monarch butterfly caterpillars feeding on MW leaves; comparison of symptomatic to nonsymptomatic leaf feeding preference.

Section III

Guidelines for Implementing Snap Bean Study

Phaseolus vulgaris, or common bean, includes our dry, field varieties—navy or pinto—and the potted garden varieties—lima or stringless. The stringless bean plant is described as having a green pod, 8 to 20 cm long, with the pod holding 4 to 12 seeds. It was selected as a bioindicator for this study because stringless bean plants show sensitivity to ozone in the form of foliar injury. Originating in the hot regions of the Americas, India and China, this vegetable became widespread in the 19th century and is frequently called the "snap bean" because, when fresh from the garden, the beans make a crisp snapping sound when being prepared for cooking.

Structure and Growth Pattern of Snap Beans

As with most seeds, the dried snap bean seed consists of a small embryo and the two obvious halves of the seed that contain the stored reserves (protein, fat, carbohydrates) that will serve as "food" for a young seedling. When the seed becomes hydrated through water absorption (imbibition), germination begins with the root radical and an immature shoot (hypocotyl) growing out of the embryo. The first leaves (cotyledons) originate from the plumule (first terminal bud of the embryo). The endosperm (two seed halves) is the food supply that swells and converts metabolically to provide energy for initial growth.

The bean seed germinates within a few days of planting in soil. During the first 3 days it collects nutrients, then the radicle or primary main root is established. The root elongates and the seed splits open to allow the young plumule and elongating shoot to emerge in about 6 days.

At about 6 days cotyledons (seed leaves) appear above the soil's surface. They will mature into 2 single foliate leaves.

During its first week of growth the snap bean plant develops 2 single foliate leaves and has the 2 cotyledons still attached to the main stem.

About 2 weeks after the single foliate leaves have matured, a new leaf begins to grow. This trifoliate (compound) leaf is unlike the original two leaves; when the trifoliate leaf opens, it will have 3 small leaflets. The 3 small leaflets make up the compound leaf.

The small short stems branching from each leaflet meet at a single point, or petiole, which is a slim leaf stem connecting the trifoliate leaf to the bean plant's main stem.

Later, when labeling leaves for symptom observation, the first trifoliate leaf will be labeled with a tag and numbered SB1-1. The tag will identify that this leaf was the first trifoliate leaf to appear on the ozone sensitive (SB) snap bean cultivar in pot #1. The first leaf is coded SB1-1 and each additional trifoliate leaf is labeled: SB1-2, SB1-3, etc., in the order they emerge along the main stem. A second trifoliate leaf is beginning to grow on the stem's left side just barely above the petiole of trifoliate leaf SB1-1.

Validation of Ozone-Induced Foliar Injury in Snap Beans

Fumigation Chamber. The fumigation chambers in the picture are used to expose sensitive snap bean plants to ozone concentrations over time and observe the results.

A USDA-ARS, 9-day study summarized the responses observed on snap bean leaves when exposed to controlled concentrations of ozone. Ozone-induced injury appeared within 1 day of exposure to ozone air pollution and increased significantly within 3, 6, and 9 days of exposure (Burkey et al., 2005).

Sensative Bean Plant
1st Leaf - Exposed One Day to Ozone

Leaf S156-1#1 has been exposed to ozone air pollution and shows some ozone-induced foliar injury after 1 day of exposure.
K. Burkey, USDA-ARS

Sensative Bean Plant
1st Leaf - Exposed Three Days to Ozone

Leaf S156-1#1 has been exposed to ozone air pollution for 3 days and shows increased injury
K. Burkey, USDA-ARS

Sensative Bean Plant
1st Leaf - Exposed Six Days to Ozone

Leaf S156-1#1 has been exposed to ozone air pollution for 6 days and shows increased ozone-induced foliar injury.
K. Burkey, USDA-ARS

Sensative Bean Plant
1st Leaf - Exposed Nine Days to Ozone

Leaf S156-1#1 has been exposed to ozone air pollution for 9 days. The stippling or purpleing color has become more pronounced. observe the veins clarity. Stippling does on occur on the veins.
K. Burkey, USDA-ARS

Visible Symptoms in the Field. Ozone-induced symptoms as observed on most common bean varieties are exhibited as a typical upper-leaf stipple with the veins remaining free of any symptoms. At first, stippling is usually light brown in color but then becomes darker shades of brown and even takes on a purple hue. Older leaves show the symptoms first with affected leaves also becoming yellow (chlorosis); the more sensitive varieties may suffer defoliation of the oldest leaves soon after late season, severe ozone episodes.

Note: It is important to look at <u>all three leaflets</u> of the trifoliate leaf when estimating the percentage of ozone-induced injury.

The snap bean cultivars begin the summer season with no ozone-induced injury (Class 1).

As the ozone concentrations increase, the trifoliate leaf will begin to show initial symptoms of ozone-induced foliar injury. The trifoliate leaf has developed initial ozone-induced stipple with the estimated rating being 1 to 6 percent (Class 2).

Ozone-induced stipple on the snap bean leaf to the left shows typical upper surface stippling. Note the clarity of the tissues along the immediate vein areas on the uppermost leaflet. The entire trifoliate leaf would be evaluated and therefore, when estimating the percentage of injury, a composite rating in the 7 to 25 percent range (Class 3) is appropriately recorded for the trifoliate leaf.

As ozone concentrations increase during the summer season, the ozone-induced foliar injury increases. The percent of injury on the trifoliate leaf in the picture to the left is 26 to 50 percent (Class 4). The leaflet to the right on the trifoliate leaf has much less injury than the other two leaflets.

Advanced ozone-induced foliar injury is shown on the snap bean leaflet in the picture to the left. The other two leaflets behind it also reveal advanced injury of 51 to 75 percent (Class 5). Careful observation reveals some small green areas.

Extreme ozone-induced foliar injury on the leaflets of the trifoliate leaf in this picture is rated at 76 to 100 percent (Class 6). Look carefully at the leaflet behind the one being held. The leaflet has senesced (started to die) from exposure to long-term high concentration ozone exposures.

Note the older leaves within the inner areas of several plants in this picture are most severely injured. The trifoliate leaf showing the most injury would be scored in the 26 to 50 percent range (Class 4). Also note that the trifoliate leaf in the upper left is not typical of ozone exposures because the injury involves the vein tissues. This trifoliate leaf would be assessed as 0-percent ozone-induced injury, but notations of the atypical symptoms would be made in the note section of the data sheet.

Following a season-long exposure to ozone air pollution, the sensitive bean plants now show abundant stipple and defoliation. The tolerant beans are just beginning to show late-season ozone-induced injury.

Mimicking Symptoms in Beans. Beans of all varieties have numerous diseases and insect caused problems with symptoms that may mimic those caused by ozone air pollution exposures. Many of these are quite common and with careful observation are easily distinguished from those caused by ozone. Observers are reminded to carefully review the very specific symptoms of ozone-induced injury when encountering the symptoms described in the following photos. Additional image details are available from the Plant Diagnostic Information System (PDIS) Image Library for use in educational materials, presentations, extension Web sites and publications, and federal, state, and local government agencies (www.pdis.org/ImageLibrary/ImageSearch.aspx).

Howard F. Schwartz,
Colorado State University, PDIS

Bean mosaic virus infection with yellowing (chlorosis) has occurred in a "mosaic" pattern. The yellowing due to ozone exposures is more uniform in appearance across the surfaces of the older leaves.

Howard F. Schwartz,
Colorado State University, PDIS

The initial symptoms of bean rust appear as chlorotic spotting, but careful observations of the lower leaf surface also show bi-facial yellowing and pustules of the causal fungus.

Howard F. Schwartz,
Colorado State University, PDIS

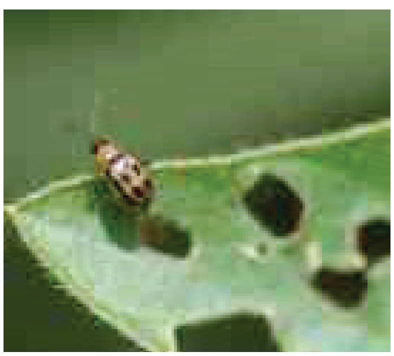
Bean rust appears with an upper surface browning in the late season. Observers must turn the leaves over to look for the pustules of the causal fungus.

K. Cardell, Brazil, PDIS

Pustules of the bean rust fungus appear on the underside of the leaf and assist in determining that the top leaf symptoms are **not** caused by ozone air pollution.

Howard F. Schwartz,
Colorado State University, PDIS

Aphids appear as small insects usually on the lower leaf surface. Their piercing of the leaf surface while feeding produces chlorotic spots and eventual browning of the upper leaf surface. Very small spider mites cause similar injury and careful observations must be completed to insure accurate diagnosis: a 10x magnifying glass is often quite helpful.



W. Upham
Kansas State University, PDIS

The bean leaf beetle is feeding on the common bean. The insect is very common and easy to recognize, as is the leaf injury.

Leafhopper adults and nymph stages injure bean plants. The adults feed by “sucking” water and nutrients from the bean leaf. The first symptoms of their presence are pale leaf veins and curled leaves (additional IPM images are available at www.bugwood.org/index.cfm).

It doesn't take long for the bean leaf hopper to injure the bean. The final injury to the leaf is dark brown raised spots that show on the upper and underside of the leaf. The severe injury caused by the leafhopper is not easily confused with ozone-induced foliar injury. The injury is noted, and the observer should carefully examine if any stipple is present. No presence of ozone stipple is rated Class 1 or 0 percent.

The upper leaf surface of the bean leaf with injury is from an unknown cause. Observers will see many similar symptoms that are not caused by ozone. In this case, the injury is expressed in large contiguous lesions with irregular borders and some with brown and dying centers. Most likely, if this leaf were turned over, the underside would also show lesions all the way through the leaf. These symptoms should be noted for the record but not scored as ozone-induced injury.

White mold develops on the bean plant during or after the flowering period. It affects the tissue of the bean pod, leaves, and stems and destroys the plant. The fungus grows well in cool, moist weather, high humidity, and when the leaves are wet for extended periods of time.

Planting Snap Beans

Establishing an ozone garden using sensitive and tolerant snap beans may be accomplished using one of two methods. The snap bean may be planted in nursery pots or freshly tilled field soil. It is important to: 1) follow the guidelines for the planting method selected, 2) provide consistent watering of the plants without over-watering them, and 3) locate the plants in a sunny area. Either method of planting the seeds provides plants that can be observed for ozone-induced foliar injury.

Planting in Pots. Planting snap beans in pots provides a uniform method for comparative studies across numerous participating programs. The following materials can be obtained at local nurseries or home and garden stores and will be needed for planting in nursery pots:

1. **12-inch diameter, 9-inch deep plant pot:** Nurseries often have old pots that they no longer use and will donate them or charge a minimum amount of money for them, or will sell new ones at minimum cost. If using old nursery pots, they must soak overnight in a bleach solution made of 4 cups bleach to 15 gallons of water. Rinse the used pots well with clean water after disinfecting. New pots do not require sterilization.
2. **Expert Gardener® Perfect Mix™**: or other potting mix composed of sphagnum peat moss and sedge peat, composted forest products, horticultural perlite, a wetting agent and lime for proper pH balance (no topsoil in mix). The mix has a plant formula that will support an excellent growing environment. Nitrogen, phosphate, and potash have been coated for slow release of nutrients. The following nutrients should be in any mix used to plant the bean seeds. Numbers are percentages.

a) Nitrogen (N)	0.11
b) Phosphate (P_2O_5)	0.04
c) Calcium (Ca)	0.01
d) Magnesium (Mg)	0.01
e) Sulfur (S)	0.04
f) Copper (Cu)	0.001
g) Iron (Fe)	0.02
h) Manganese (Mn)	0.004
i) Zinc (Zn)	0.002

3. **Osmocote®:** a slow time-release fertilizer that will provide plant nutrition up to 4 months.
4. **Reflectix®:** insulating material affixed to the outside of pots to minimize temperature changes to roots between day and night.
5. **3-foot wooden stakes:** one for each bean plant.
6. **Standard or soaker hose:** for watering the plants.
7. **Pot label stakes.**
8. **Black permanent marker to label pots and or stakes.**
9. **Small tags with cotton string attached:** (available at office supply stores) label each leaf as it emerges on the main stem of the plant.
10. **10x magnifying glass:** observe the plants for the first symptoms of stippling or purpling of the leaves.
11. **Paper towels.**
12. **Digital camera:** to photograph plants and leaves as they grow and respond to ozone air pollution.

Procedures. The following procedures are for planting snap beans in pots:

1. Cover the holes in the bottom of each pot with a single layer of paper towel to keep the mix from falling out of the pot.
2. Wrap the outside of each pot with a single layer of Reflectix®, an insulating foil wrap to keep the pots cool and therefore having less evaporation of water from the soil surface. Fill each pot three quarters full with Expert Gardener® Perfect Mix™ .
3. Add one-quarter cup Osmocote® to each large pot and mix well into the potting mix using bare hands.
4. Add more potting mix to fill the pot to within about 1 inch from rim
5. Saturate each pot with tap water before planting to hydrate the nutrient rich potting mix.
6. Place the pots in a sunny area and allow to drain to soil moisture holding capacity, i.e., no further draining of water is occurring
7. Using a permanent marker, label the stake within each pot to identify the type of bean that will be planted in it. For example, if the pot will be used to plant ***T****olerant* snap beans, label the pot ***T***. If there is to be a second pot planted with tolerant snap bean seeds, then the first pot ID is TB1 and the second pot is labeled TB2. The pot to be used for planting ***S****ensitive* bean seeds is labeled ***S***, and if more than one pot is prepared for sensitive bean seeds, then each pot will be labeled SB1, SB2, and continued for each additional bean plant.

8. Before planting any bean seeds in the newly labeled pots, water each pot once again to prepare them to receive the bean seeds. Once each pot is very moist, plant three tolerant bean seeds in the pot with the ID TB1 about 2 cm apart and at a 2-cm depth in the center. Cover and gently wet the surface of the mix to get good soil contact with the seeds. Repeat this process using ozone sensitive snap beans for the pot with the ID SB1. Continue the process for the rest of the pots to be used for planting tolerant and sensitive snap beans.
9. Approximately 10 days after planting there should be three young bean plants with two single leaves (unifoliate leaves). Snip off two of the plants leaving the strongest plant to grow in the pot. Transplanting the two young seedlings rather than snipping them off does not usually work due to plant shock before roots can re-establish. In addition, pulling the weaker seedlings may damage the tender roots of the seedling selected for the study.
10. Water the plants as needed. It is better to overwater because the potting mix is designed to drain well. It is important to keep the soil moist but not saturated. **CAUTION:** Do not use a watering dish under the plants because it will cause water logging of the soil and lead to rot of the plant's roots**.**

Planting in Tilled Soil. Planting the bean seeds in a garden area is an alternative to planting them in pots. This enables planting several seeds of each cultivar. It is extremely important to have the stakes in-ground to identify the bean plants as tolerant (T) or as sensitive (S) cultivars.

1. Expert Gardner® Perfect Mix™ : or other potting mix composed of sphagnum peat moss and sedge peat, composted forest products, horticultural perlite, a wetting agent and lime for proper pH balance (no topsoil in mix). The mix has a plant formula that will support an excellent growing environment. Nitrogen, phosphate, and potash have been coated for slow release of nutrients. The nutrients necessary for planting in soil are the same as that listed for planting in pots.
2. **Osmocote®:** a slow time-release fertilizer that will provide plant nutrition up to four months.
3. **3-foot wooden stakes:** one for each bean plant.
4. **Standard or soaker hose:** for watering the plants.
5. **Digital camera:** to photograph plants and leaves as they grow and respond to ozone air pollution.

6. **Black permanent marker to label pots and or stakes.**
7. **Small tags:** label each leaf as it opens on the main stem of the plant.
8. **10x magnifying glass:** observe the plants for the first symptoms of stippling or purpling of the leaves.

A question might be raised at this point as to why the same materials used to plant bean seeds in a nursery pot are used to plant the seeds in tilled soiled. Following this procedure will provide consistent planting environments regardless of the method used. If one site wants to compare potted plants with those planted in tilled soil, the planting medium and process will be more or less consistent between processes.

Consistency Between Planting Methods. To maintain consistency in planting the bean seeds in pots or in tilled soil, the same potting soil and Osmocote® mixture is used for planting the snap bean seeds. However, for planting directly in the garden:

1. Select a sunny area and dig eight holes 30 cm (12 in.) wide and 23 cm (9 in.) deep, and remove the natural soil to another part of the garden.
2. Use a 30 cm (12 in.) diameter by 23 cm (9 in.) deep pot to mix the soil with the Osmocote®. Add one-quarter cup of Osmocote® into the potting mix using your hands and then pour the mixture into the hole in the ground.
3. Fill each hole to the top with potting mix mixed with Osmocote®: Flush the soil in each hole with tap water to hydrate the nutrient rich mix.
4. Place 2 to 3 bean seeds of the same cultivar, either sensitive or tolerant, in a close circle in the new soil, about 5 to 7 cm (2 to 3 in.) apart.
5. Cover the seeds with the potting mix.
6. It is also very important that the stakes have been placed at each bean plant site and marked to identify whether they are tolerant (T) or sensitive (S) bean plants. Label the sensitive bean plant sites SB1, SB2, and SB3 and SB4, and the tolerant plants TB1, TB2, TB3, and TB4.

Assessing Ozone-Induced Foliar Injury to Snap Beans

Depending upon when the bean seeds were planted, and upon local weather conditions (and, of course, ozone exposure), the first assessment of ozone-induced foliar injury should occur by mid-June. The final assessment of plant injury should be before the first hard frost in the area of the study.

Labeling Plants and Leaves for Observation. The plant stakes can easily be moved either inside or away from the site by vandals. Therefore, it is also important to tag the young bean plants as soon as they break through the ground.

1. Once plants emerge, water them.
2. Approximately 10 days after planting, each bean plant should be about 2 to 3 in. tall. Select one to keep and snip the tops off the other two plants that grew in the same site. Transplanting the two young seedlings does not usually work due to plant shock before its roots can re-establish. Pulling the seedlings from the soil may damage the tender roots of the seedling selected for the study.
3. Put a large tag at the base of the bean plant to identify it as TB1 and repeat the process for the other tolerant and sensitive bean plants (TB2, TB3 and TB4, and for sensitive plants SB1, SB2, SB3 and SB4).
4. Begin tagging the trifoliate leaves as they appear on the stem with a small paper tag tied to the leaf petiole with a cotton string. The first tolerant snap bean plant will have the ID TB1; the first leaf will be labeled TB1-1. The leaf sequence will be: TB1-1, TB1-2, TB1-3, etc., up to the tenth leaf. For the second tolerant plant the ID TB2 will precede the leaf number. This identifies it as leaf #1 on the second tolerant plant. The leaves on all four tolerant plants will be tagged following the same pattern. The leaves on the sensitive plants will be tagged SB1-1, SB1-2, SB1-3, etc., up to the tenth leaf. The second plant will be tagged SB2-1, SB2-2, SB2-3, etc., up to the tenth leaf. The last two plants will follow the same leaf numbering pattern using the specific plant ID code. The data sheet for each bean plant is set up with the specific plant ID and leaf code. Be sure the plant matches the data sheet when collecting data.
5. Label each new leaf as it emerges on the plant.

Materials Needed for Plant Observation. A variety of materials are needed in preparation for assessing foliar injury. Organizing the needed materials in a container to consistently transport them to the area where the bean plants are growing will simplify the task.

1. A meter stick to measure the height of the plant; a small flat board will assist in making height measurements when placed under the meter stick for stability on the soil surface
2. A 10x magnifying glass to initially find the stippling and carefully examine leaves for small insects and presence of fungus.
3. Snap bean field guide.

4. Small tags with string, and black fine point marker to label new trifoliate leaves.
5. Clipboard with data sheets and pencil.
6. Digital camera for periodically recording the progression of symptoms.

Plant Assessment Procedures: Ozone-Induced Effects. Using a 10x magnifying glass enables the observer to see the beginning of stippling. Ozone-induced foliar injury may become visible by mid-June and more likely even later during the summer season. Beware of mimicking symptoms and other pest problems that may produce symptoms that look like ozone air pollution injury to sensitive bean plants. One strategy is to look at older leaves as they should have the most injury. If younger leaves have the most injury, it most likely is not ozone induced. The stippling should be on the upper leaf surface of the leaf only and not on the underside. Also, stippling does not affect or appear on any of the leaf's veins. For some species, the stippling is called purpling because it looks like fine, deep purple dots. On the snap bean plant, however, the stippling is a tan to light tan color.

Use the following steps to assess ozone-induced foliar injury and record your data on the data sheet for either the tolerant cultivar or sensitive cultivar. Use a different data sheet for each plant. During each observation period:

1. Measure the plant's height from the base to the apex using a meter stick and record the height in centimeters. Place the meter stick on a small piece of wood to keep it from depressing into the ground. Measure the plant's height from the base of the plant to the base of the petiole of the highest leaf.
2. Only the 10 bottom leaves are numbered and if, after labeling, a leaf falls off near the bottom of the plant, it is still counted as one of the original ten leaves used for data gathering. Each week carry the last data forward that was previously recorded for the fallen leaf.
3. Assessing injury to a trifoliate leaf may be done one of two ways:
   a. Look at one leaflet at a time and estimate the injury to each leaflet of the trifoliate leaf. Add the three estimates and find the average injury to the <u>whole</u> leaf. Record the average.
   b. Look at the whole leaf and try to estimate the percentage of injury.

4. Each leaf is rated for:
   a. S = Stippling (most commonly tan colored stipple on the upper leaf surface <u>only</u>).
   b. C = Chlorosis (yellowing of the leaf indicating loss of chlorophyll in cells).
   c. N = Necrosis (portions of leaves that are dead and usually brown in color).

5. Estimating the percentage of injury to a leaf requires practice. To practice and develop this skill, use the resources found at: **www.ozonegarden.larc.nasa.gov**

The ratings are given along with their percentage values. For example, if the plant has no ozone-induced foliar injury, it would be given a rating of Class 1, whereas if it shows a few symptoms a rating of Class 2. The range of six rating classes used to assess ozone induced foliar injury are:

Class 1 =	0%	No ozone-induced injury
Class 2 =	1 to 6%	Light ozone-induced injury
Class 3 =	7 to 25%	Moderate ozone-induced injury
Class 4 =	26 to 50%	Moderately severe ozone-induced injury
Class 5 =	51 to 75%	Severe ozone-induced injury
Class 6 =	76 to 100%	Extremely severe ozone-induced injury

1. Plants should be assessed for symptoms only on bright sunny days and with the sunlight over the observer's shoulder. Observations for symptoms should be continued at 2 to 3 day intervals, until the first symptoms of typical ozone-induced injury appear. Continue assessing the plants once a week, on the same day of week or as close as may be possible, providing there are sunny conditions.
2. Record the number of flowers and then the number of seedpods per plant as they develop near the season's end.
3. When a leaf falls off, carry its last data entry over into all of the ensuing data collection entries. This is to show leaf loss on a graph.
4. If insects eat part of a leaf or if various diseases appear, rate only the surface you can see for ozone-induced injury and identify the percentage of leaf missing.

As the exposure to ozone levels increases, foliar injury will increase. Also, a leaf may simply break off the stem. If the leaf is still present on the ground at the base of the plant, and preferably with the paper tag attached, look at the dropped leaf and assign it the appropriate leaf loss code using the following criteria:

Class 7 = leaf dropped with no prior symptoms or just missing
Class 8 = leaf dropped with prior chlorosis (yellowing) only
Class 9 = leaf dropped with prior stippling (the tan to brownish dots) only
Class 10 = leaf dropped with all or some of the following: stippling (small dark colored dots), chlorosis (yellowing), or necrosis (browning)

Never make assumptions about what you are seeing on the leaf. Just rate what you see at that particular point in time. Insects will also eat part of some leaves during the season. Rate the leaves on what is visible, and note in the comments area for that leaf the percentage of the total leaf missing. Stop collecting data once the plants have been through their first frost event of the late summer or fall season.

Filling in the Data Sheet. Data sheets have been designed for recording the percentage of ozone-induced foliar injury on the first ten leaves of each bean plant. Note that a plant **ID number must be recorded** at the top of each data sheet. The code for four tolerant snap bean plants are identified: TB1, TB2, TB3, and TB4. The code for four sensitive snap bean plants are identified: SB1, SB2, SB3, and SB4 . Each single plant data sheet is for gathering plant data for one day on a specific plant. Specific information about the observer and the plant is to be recorded at the top of each data sheet. The observer may also indicate the type and location of injury on each leaf drawn on the data sheet starting from the oldest leaf 1# at the bottom of the data sheet and work upward to the top of the plant.

Additional Activities. In addition to observing plant injury, the yield from each plant may be analyzed by:

- Identifying the quantity of bean pods produced for each plant.
- Identifying the average bean pod length for sensitive and tolerant beans.
- Opening the bean pods and determining the number of beans produced and their weight (yield measurements).
- Gently removing the dried plant and roots, rinsing and drying off the roots of tolerant and sensitive bean plants, and weighing each plant.
- Comparing the root ball sizes between the sensitive and tolerant bean plants.
- Taking photos of injured leaves during each observation period and keeping a photo journal of each plant and the selected leaves being observed.

Snap Bean Field Guide

0%   Leaf Injury = Level 1

Rating System:
1 = 0% injury
2 = 1-6% injury
3 = 7-25% injury
4 = 26-50% injury
5 = 51-75% injury (leaf has very small green areas
6 = 76-100% injury (Leaf has no small green areas)

1-6% Leaf Injury = Level 2

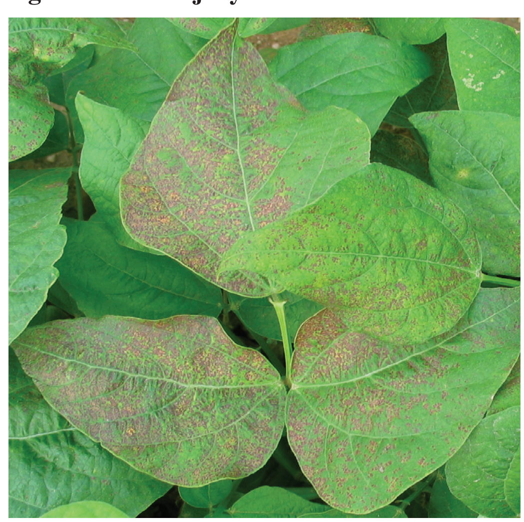
Lower end of injury Level

Higher End of Injury Level

7-25% Leaf Injury = Level 3

Lower end of injury Level

Higher End of Injury Level



26-50% Leaf Injury = Level 4

Lower end of injury Level

Higher End of Injury Level

51-75% Leaf Injury = Level 5

Lower end of injury Level

Higher End of Injury Level

76-100% Leaf Injury = Level 6

Lower end of injury Level

Higher End of Injury Level

Bean Injury NOT Ozone Induced

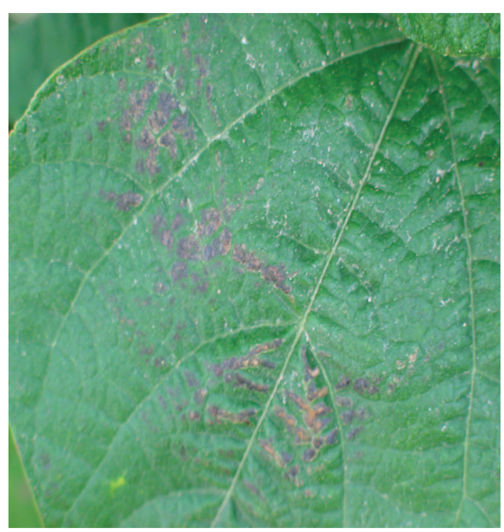
Bean Mosaic

Bean Leaf Hopper Injury

Bean Rust

Bean Rust with Chlorotic Spots

Atypical symptoms of unknown cause but NOT Ozone injury



Atypical symptoms of unknown cause but NOT Ozone injury

Snapbean Plant ID= R331#1

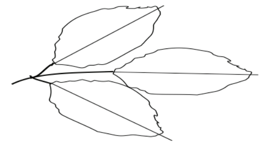
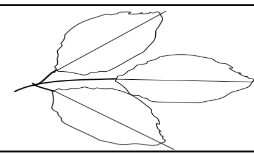
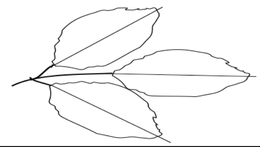
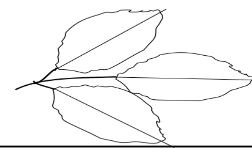
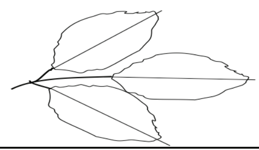
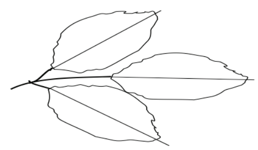
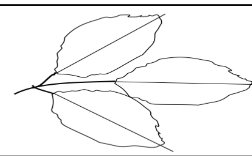
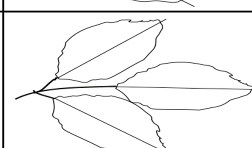
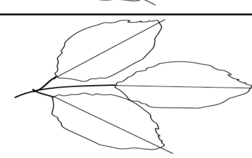
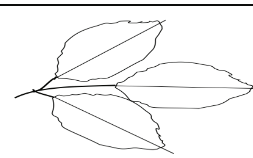
Data Sheet

Name: ________________________________ Date:

Site Name: ____________________________ Plant Height cm:_______ # of leaves on plant:_______

leaves showing ozone injury:__________ # of Seed Pods: __________

Directions: This sheet is set up to observe, record data and create a color model of the first 10 leaves. Please record data, and then color each leaf as it appears in field using colored pencils: Ozone= black; Chlorosis=yellow and Necrosis=brown.

Plant ID & Leaf Tag No.	% Ozone Injury	% Chlorosis (yellowing)	% Necrosis (browning)	% Dead leaf (senesced)		Field Notes
R331#1-10						
R331#1-9						
R331#1-8						
R331#1-7						
R331#1-6						
R331#1-5						
R331#1-4						
R331#1-3						
R331#1-2						
R331#1-1						

Section IV

Guidelines for Implementing Cutleaf Coneflower Study

The cutleaf coneflower *(Rudbeckia laciniata)* is a native perennial plant found throughout most of the U.S. The species spreads naturally by seed and from long spreading rhizomes. The cutleaf coneflower is ever present in the Great Smoky Mountains National Park (GSMNP). As part of a long-term investigation of plant ecology at Great Smoky Mountains National Park, the cutleaf coneflower has been evaluated for ozone-induced foliar injury.

Validation of Ozone-Induced Foliar Injury on Cutleaf Coneflowers

Fumigation Experiments. A study was set up using open-top chambers at the Twin Creeks facility at Great Smoky Mountains National Park in 1987. From 1988 to 1992, nearly 40 different plant species were fumigated with ozone of differing concentrations, ranging from charcoal-filtered air-supplied chambers (which scrub out most of the ozone) to ambient concentrations (same as in the ambient air outside the chambers), and also with ozone added to 50 and 100 percent above ambient levels (Neufeld et al., 1992). A variety of species, including both trees and wildflowers, were exposed to ozone over the course of two seasons. The aim of these studies was to verify that the ozone-like symptoms seen in the field on these species was indeed due to ozone exposures. Thirty of the forty species that were grown and fumigated in the chambers showed ozone-like symptoms after being exposed to elevated ozone. Thus, the researchers were confident that the symptoms they were identifying in the field were due to ozone and not other factors.

Cutleaf coneflowers were exposed over two consecutive growing seasons. For most cutleaf coneflower plants that were sensitive to ozone, growth was reduced whenever the ozone was elevated above ambient air levels (the elevated ozone treatment levels of 50 and 100 percent). After the first season, there were no effects on either leaf or flower weight, but ozone-induced foliar stipple was greatly accentuated in the higher ozone exposure treatments. Although the researchers could not detect any growth effects, it was apparent that the leaves were highly sensitive to elevated ozone. Other species, such as black cherry, yellow poplar, and whorled wood aster had significant growth reductions in the elevated ozone treatments.

In The Field. Field research included four sampling periods for ozone-induced injury at approximately 3- to 5-week intervals from June to September during 2000. Cutleaf coneflowers were examined on and off trail (off-trail plants were more that 5 m from the trail) at Purchase Knob and Clingmans Dome in the Great Smoky Mountains National Park.

The level of ozone-induced injury was assessed using two methods: 1) the number of injured leaves per plant, and 2) the percentage of leaf area injured. The findings of the study at Clingmans Dome trail identified 50 percent of the overall population of the plants studied had ozone-induced injury. Plants growing near the trail had significantly greater injury and 3.5 times greater leaf area injury than those growing off trail. The leaves on the lower half of the plants had 95 percent of the leaf injured. The pattern of injury (older leaves most severely injured) was similar for plants near and off trail.

The results with on- and off-trail plants at Purchase Knob showed no differences in the level of injury between the two groups of plants. However, ozone-induced injury was greater for the Clingmans Dome plants than for those at Purchase Knob (Chappelka et al., 2003).

The cutleaf coneflower, *Rudbeckia laciniata*, is a flowering plant with leaves and stems that die at the end of the growing season. During the next spring season a new coneflower will arise from the underground roots called "rhizomes." As illustrated here, seeds from sensitive cutleaf coneflower plants may be covered to keep them from being eaten by birds, and they may be dried and saved for future planting. Only the seeds and rhizomes from verified ozone- sensitive parent plants should be used for this project. (Sources of verified seeds may be found on the acknowledgment page.)

An observer needs to begin looking at healthy cutleaf coneflower leaves, as in this leaf, with 0-percent stipple (Class 1), from mid-June through the remainder of the summer depending upon the geographic region and when season-long exposures to ozone begin to increase. A 10x magnifying glass will be very useful to correctly identify the initial very fine ozone-induced stipple.

As the ozone season begins and ozone concentrations increase, the older leaves of the cutleaf coneflower will likely begin to show symptoms of ozone-induced stipple. The amount of stipple present on in this cutleaf coneflower leaf is between 1 to 6 percent (Class 2).

The development of ozone-induced foliar injury progresses on the leaf's surface as ozone concentrations increase. This leaf is beginning to show increased injury between 7 to 25 percent (Class 3).

The ozone-induced stipple on the cutleaf coneflower leaf shows typical **upper** surface injury. Note the clarity of the tissues along the immediate vein areas on the upper surface and large green areas are still present. This leaf shows injury between 26 to 50 percent (Class 4).

Increased ozone concentrations for extended periods of time will increase the severity of ozone stipple. There are small areas of green leaf tissue still present on the leaf's surface. The leaf is showing advanced ozone-induced foliar injury between 51 to 75 percent (Class 5).

The extreme ozone-induced foliar injury in the cutleaf coneflower leaf here is estimated between 76 to 100 percent (Class 6).

Mimicking Symptoms on Cutleaf Coneflower. Not all injury seen on the cutleaf coneflower is ozone induced. The cutleaf coneflower is susceptible to fungal, bacterial, and insect injury. When the injury is on both the upper and under side of the leaf, it is **not** ozone-induced stipple. Careful observations will distinguish injury caused from ozone air pollution and symptoms caused by other sources. When in doubt, submit a picture of the injury to the NASA Web site www.ozonegarden.larc.nasa.gov for professional assistance.

Additional diagnostic information about the cutleaf coneflower may be found at: www.mobot.org/gardeninghelp/plantfinder/IPM.asp?code=263&group=39&level=s

This leaf shows a small track of a leafminer larva eating inside the cutleaf coneflower foliage. Note other necrotic (brown) injury is crossing over vein tissue and therefore not ozone induced.

White or gray powdery mildew is commonly found on cutleaf coneflower. It develops during periods of high relative humidity and on plants growing too close to each other.

Planting the Cutleaf Coneflower Seeds

Planting in Tilled Soil. The natural soil (topsoil) in the garden is used for planting cutleaf coneflower seeds. Till the soil within the garden area that will be used for planting the seeds and remove all weeds to provide an easy environment for the young plants to germinate and grow.

Poke 2 to 3 holes about 2 cm (1/2 in.) deep in a small circle using your finger. Drop a coneflower seed in each hole and cover lightly. Mist the surface soil if it is not moist from earlier watering. Create three more areas about 36 cm (18 in.) apart using the same process. When the young plants emerge, keep the strongest plant in each section and carefully cut off the extra seedlings at the ground line to allow the single plant to grow for data gathering.

Planting Coneflower Seeds in Pots. Some participating groups may not have room for a establishing a complete in-ground bioindicator garden. The cutleaf coneflower is an excellent choice for planting in large pots filled with local soil that will support an extensive root system. Commercially available pots from nurseries made of clay and or wood products work best. Large black plastic pots likely will become too hot and the plant's root system will dry out quickly. Grow a single plant in each pot, place it in a sunny area, and be sure to water it daily, if needed. Keep the soil moist, but not too wet, to prevent water stagnation and rotting of the roots.

Coneflowers perform best if planted in the early fall so they have a chance to establish themselves before winter. In warmer climates, coneflower will develop a rosette of basal leaves that remain green in the winter as the main stem with leaves dies. The plant will grow a new stock in the center of the rosette in the spring. The emerging new stem will produce buds and new leaves. These new leaves are the part of the plant used to assess ozone-induced injury, not the ground level leaves of the rosette.

Organizing Plants for Observation. As the plant begins to grow, gently place a 1.5-m (about 4 ft) stake 30 cm (1 ft) into the ground and put the plant's ID on the stake. Place a tag with a sturdy plastic tie loosely at the base of the plant to be evaluated, the additional tag will enable identifying the plant's ID in the event the stake gets broken or removed.

Assessing Plant Injury to Cutleaf Coneflowers

Labeling Plants and Leaves for Observation. Once the design of the garden has been established and coneflower seeds planted, it will take about a 2-year season for the cutleaf coneflower plant to reach robust full growth. When the plants have reached full growth, then each plant will need to have an ID number using CF for cutleaf coneflower. Use the following numbering pattern to ID each cutleaf coneflower plant. Select only four cutleaf coneflower plants for the initial monitoring period. ID the first plant as CF1, a second plant CF2, a third plant CF3, and the fourth plant CF4. This will help prevent confusing data taken from one coneflower plant with another. Eventually a rosette of leaves will grow at the base of the plant, so be sure the individual ID stakes are taller than the rosette. It is important that the plant ID number and leaf labeling pattern be developed during the initial set up of the garden to identify the same cutleaf coneflower each year for taking measurements.

After each plant has an ID, the observer will need to identify and label each leaf that will be assessed for ozone-induced foliar injury.

- **Starting from the bottom of the stem,** the first leaf is tagged with a loosely tied string attached to a tag (tags available at office supply stores) as #1, then move in a clockwise fashion up the stem. Label the next leaf in sequence #2 and continue numbering the 10 leaves as they emerge during the early growing season (e.g., CF1-1, CF1-2, etc.).

- You may use a black permanent marker and very gently write (very small) the leaf number on the tip of the underside of the leaf. This should be done carefully to avoid damaging the leaf and is important because the plant twists as it grows and this measure will ensure each data gatherer is collecting data on the <u>same</u> leaf.

Materials Needed for Plant Observation. A variety of materials are needed in preparation for assessing foliar injury. Organizing the needed materials in a container to consistently transport them to the area where the bean plants are growing will simplify the task.

1. A meter stick to measure the height of the plant; a small flat board will assist in making height measurements when placed under the meter stick for stability on the soil surface.

2. A 10x magnifying glass to initially find the stippling and carefully examine leaves for small insects and presence of fungus.
3. Cutleaf coneflower field guide.
4. Small tags with string, and black fine point marker to label new leaves.
5. Clipboard with data sheets and pencil.
6. Digital camera for periodically recording the progression of symptoms.

Plant Assessment Procedures: Ozone-Induced Effects. Using a 10x magnifying glass enables the observer to see the beginning of stippling. Ozone-induced foliar injury may become visible by mid-June and more likely even later during the summer season. Beware of mimicking symptoms and other pest problems that may produce symptoms that look like ozone air pollution injury to cutleaf coneflower plants. One strategy is to look at older leaves as they should have the most injury. If younger leaves have the most injury, it most likely isn't ozone induced. The stippling should be on the upper leaf surface of the leaf only and not on the underside. Also, stippling does not affect or appear on any of the leaf's veins. The stippling on the cutleaf coneflower is called purpling because it looks like fine, deep purple dots.

Use the following steps to assess ozone-induced foliar injury and record your data on the data sheet for either the tolerant cultivar or sensitive cultivar. Use a different data sheet for each plant.

During each observation period:

1. Measure the plant's height from base to apex using a meter stick and record the height in centimeters. Place the meter stick on a small piece of wood to keep it from depressing into the ground. Measure the plant's height from the base of the plant to the base of the petiole of the highest leaf.
2. Only the 10 bottom leaves are numbered and if after labeling, a leaf falls off near the bottom of the plant, it is still counted as one of the original ten leaves used

for data gathering. Carry forward each week the last data that was previously recorded for the fallen leaf.

3. Look at one leaf at a time and estimate the percent of injury.
4. Each leaf is rated for:

   S = Stippling (most commonly dark purple colored stipple on the upper leaf surface only).

   C = Chlorosis (yellowing of the leaf indicating loss of chlorophyll in cells).

   N = Necrosis (portions of leaves that are dead and usually brown in color).
5. Estimating the percentage of injury to a leaf requires practice. To practice and develop this skill, use the resources found at: www.ozonegarden.larc.nasa.gov

The ratings are given along with their percentage values. For example, if the plant has no ozone-induced foliar injury, it would be given a rating of Class 1, whereas if it shows a light ozone-induced symptoms a rating of Class 2. The range of six rating classes used to assess ozone induced foliar injury are identified below:

Class 1 = 0%	No ozone-induced injury
Class 2 = 1 to 6%	Light ozone-induced injury
Class 3 = 7 to 25%	Moderate ozone-induced injury
Class 4 = 26 to 50%	Moderately severe ozone-induced injury
Class 5 = 51 to 75%	Severe ozone-induced injury
Class 6 = 76 to 100%	Extremely severe ozone-induced injury

1. Plants should be assessed for symptoms only on bright sunny days and with the sunlight over the observer’s shoulder. Observations for symptoms should be continued at 2- to 3-day intervals, until the first symptoms of typical ozone-induced injury appear. Continue assessing the plants once a week, on the same day of week or as close as may be possible, providing there are sunny conditions.
2. Record the number of flowers and then the number of seedpods per plant as they develop near the season’s end.
3. When a leaf falls off, carry its last data entry over into all of the ensuing data collection entries. This is to show leaf loss on a graph.
4. If insects eat part of a leaf or if various diseases appear, rate only the surface you can see for ozone-induced injury and identify the percentage of leaf missing.

As the exposure to ozone levels increases, foliar injury will increase. Also, a leaf may simply break off the stem. If the leaf is still present on the ground at the base of the plant, and preferably with the paper tag attached, look at the dropped leaf and assign it the appropriate leaf loss code using the following criteria:

Class 7 = leaf dropped with no prior symptoms or just missing
Class 8 = leaf dropped with prior chlorosis (yellowing) only
Class 9 = leaf dropped with prior stippling (the tan to brownish dots) only
Class 10 = leaf dropped with all or some of the following: stippling (small dark colored dots), chlorosis (yellowing), or necrosis (browning)

Never make assumptions about what you are seeing on the leaf. Just rate what you see at that particular point in time. Insects will also eat part of some leaves during the season. Rate the leaves on what is visible, and note in the comments area for that leaf the percentage of the total leaf missing. Stop collecting data once the plants have been through their first frost event of the late summer or fall season.

Filling in the Data Sheet. Data sheets have been designed for recording the percentage of ozone-induced foliar injury on the first ten leaves of each cutleaf coneflower plant. Please note that a plant **ID number must be recorded** at the top of each data sheet. The code for adding a 1 at the end of the number identifies the plant as the first coneflower plant. The ID's for the four coneflower plants are: CF1 for the first plant, CF2 for the second plant, CF3 for the third plant and CF4 for the fourth plant. The leaves on each plant are identified as 1 to 10 from the **bottom oldest leaf** to the **top youngest leaf.** Each single plant data sheet is for gathering plant data for one day on a specific plant. Specific information about the observer and the plant is to be recorded at the top of each data sheet.

Coneflower Field Guide

0% Leaf Injury = Level 1

Rating System:

1 = 0% injury
2 = 1-6% injury
3 = 7-25% injury
4 = 26-50% injury
5 = 51-75% injury (leaf has very small green areas
6 = 76-100% injury (Leaf has no small green areas)

1-6% Leaf Injury = Level 2

Lower end of injury Level

Higher End of Injury Level

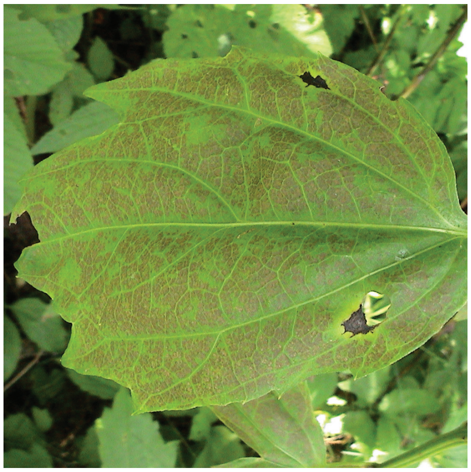
7-25% Leaf Injury = Level 3

Lower end of injury Level

Higher End of Injury Level

26-50% Leaf Injury = Level 4

Lower end of injury Level **Higher End of Injury Level**



51-75% Leaf Injury = Level 5

Lower end of injury Level **Higher End of Injury Level**

76-100% Leaf Injury = Level 6

Lower end of injury Level **Higher End of Injury Level**

Coneflower Injury NOT Ozone Induced

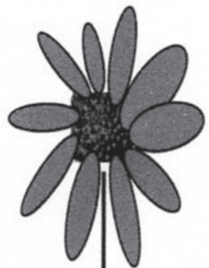
Check both the upper and under side of the leaf. If the injury goes through the leaf, it is not ozone induced.

Upper surface injury of the leaf to the right inthe picture is ozone induced; the few necrotic spots on the leaf to the leaf are NOT ozone induced. The injury goes from the surface of the leaf through to the underside.

If a leaf develops white mold that covers both leaf surfaces, it should be noted in the data sheet and no further evaluation of ozone induced injury is possible for that leaf. If ozone induced injury was present during the previous weeks of observations, that data should be carried forward.

The injury to this leaf is most likely injury from a leaf mining insect. The injury goes from the surface of the leaf through to the underside of the leaf. It is NOT ozone induced foliar injury. The other necrotic (brown) injury is crossing over vein tissue and is therefore not ozone induced.

DATA SHEET- Coneflower CF____ Date________



10

Leaf #10 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

9

Leaf #9 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

8

Leaf #8 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

7

Leaf #7 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

6

Leaf #6 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

5

Leaf #5 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

4

Leaf #4 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

3

Leaf #3 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

2

Leaf #2 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

1

Leaf #1 Leaf present ☐ Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Section V

Guidelines for Implementing Common or Tall Milkweed

Milkweed: An Herbaceous Perennial

The common milkweed *(Asclepias syriaca*) is a native perennial found throughout most of North America east of the Rockies. The species spreads naturally by seed and from long underground rhizomes, does well in sandy soils, and prospers in lots of sunlight. Milkweed species are the main source of food for the monarch butterfly, thus giving it the informal name of butterfly flower.

Historically, efforts have been made to use the common milkweed as a natural resource. During World War II the milkweed pods were gathered and the floss was used to fill life jackets. During the 1970s it was projected that synthetic crude oil could be recovered from the biomass of milkweed. This proved not to be economically feasible because the price of collection was extremely high for the low yield obtained. In 1989 the milkweed floss was used to stuff pillows for people allergic to goose down, so the effort to use milkweed as a quickly renewable resource is still being explored.

The common milkweed is an herbaceous perennial plant (has fleshy parts and withers after each growing season) that usually grows 1-to 2-m tall. New plants may grow from seed or from a rhizome defined as a thick underground horizontal stem that produces roots and shoots which develop into new plants. The surface of the main stem is very hairy and a sticky, white milky liquid is exuded (latex) when any part of the plant is injured.

The leaves are arranged in opposite pairs along the stem with each pair of leaves produced at right angles to the previous pair when following up the stem. The shape and size of the leaves may vary slightly from the much larger lower leaves at the ground to the much smaller leaves found at the very top of the plant. The leaves at the top of the plant tend to grow slightly in an upward position, making it necessary to measure the height of the plant from the ground up to the base of the petiole of the top most leaf.

Milkweed is a food source for several insects, the most commonly known being the monarch butterfly (*Danaus plexippus*). During the caterpillar stage, it feeds exclusively on milkweed. The caterpillar feeds, grows, and then develops a hard case called chrysalis. It pupates on the stems and hanging leaves of the plant during this stage. Following the pupal stage, the caterpillar then becomes an adult through a process called metamorphosis and emerges as a butterfly. Monarch Watch is an organization that follows the feeding and migration patterns of the monarch butterfly (monarchwatch.org).

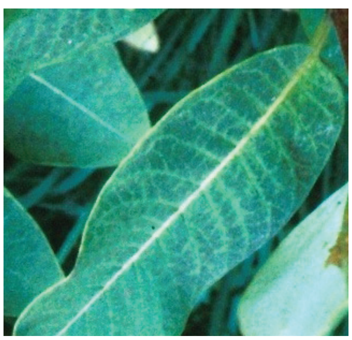
Ozone air pollution causes injury to many sensitive plants within natural plant populations. Ozone sensitive bioindicators, including common milkweed, can indicate the presence of ozone air pollution in remote areas away from more sophisticated air quality monitoring stations.

These healthy milkweed leaves have 0 percent ozone-induced foliar injury and is rated Class 1. The leaves are also insect free at this time.

As the concentrations of ozone air pollution begin to increase, the tall and common milkweed plants begin to show ozone-induced symptoms (stippling) of 1 to 6 percent (Class 2) on the upper side of the leaf.

Season long increasing exposures to ozone cause likewise increases in symptom development with this leaf showing ozone-induced foliar injury of 7 to 25 percent (Class 3).

The ozone-induced foliar injury increases, covering more of the surface of the leaf during the late summer season. This leaf is beginning to show moderate ozone-induced injury covering nearly 26 to 50 percent (Class 4) of the upper surface.



The more severely injured leaf has stippling covering all most all of the surface of the leaf and has 51- to 75-percent (Class 5) ozone-induced injury. Chlorosis and necrosis are not yet present on this highly injured leaf.

The final phase of the ozone-induced injured leaf is senescence and death of the leaf. The leaf to the left is showing very severe ozone-induced injury in the 76- to 100-percent (Class 6) range.

Validation of Ozone-Induced Foliar Injury in Milkweed

During the summer and fall seasons of the late 1970s in many areas of the Blue Ridge Mountains of Virginia, common milkweeds were observed to develop purple stippling (a discrete and very fine symptom consisting of purple colored spots on the upper leaf surfaces of the older leaves) and eventual overall chlorosis (the yellowing of the leaf caused by the loss of chlorophyll needed for photosynthesis).

The question raised was: Is surface ozone responsible for the foliar (plant) injury? Two studies were developed to determine whether long-range transported ozone air pollution within slow moving air masses into the Blue Ridge Mountains and the Shenandoah National Park were responsible for the observed symptoms.

Fumigation Experiments. A field study was initiated in the early summer of 1979 using open-top chambers within the high-elevation Big Meadows area of the Shenandoah National Park; treatments involved filtered and nonfiltered air supplied chambers and open plots in a replicated study design. By mid-June, stippling and chlorosis were observed on milkweed plants growing within the nonfiltered air-supplied open-top chambers, as well as on the milkweed growing in open plots in the field. Milkweeds growing in charcoal-filtered air supplied chambers were free of symptoms and considerably larger and greener than those in the open plots and nonfiltered air supplied chambers.

Greenhouse Evaluation. During the fall season of 1979, seeds of symptomatic milkweed plants were collected, stratified, and planted in natural field soil within a greenhouse. Eleven-week-old milkweed plants were randomly placed into Continuously Stirred Tank Reactor fumigation chambers and exposed to one of three ozone exposures: 0.00 ppb, 50 ppb, and 150 ppb of ozone for 6 hours a day for 7 days.

Foliar symptoms developed on the upper leaf surfaces of milkweed plants in the chambers within 2 to 5 days after they were fumigated (exposed) with the different levels of ozone. The milkweed plants in the chambers with the higher ozone exposures demonstrated increased stippling on the upper leaf surfaces of the older leaves; similar symptoms eventually developed on milkweeds in the 50 ppb exposures but with less severity as would be expected (Duchelle and Skelly, 1981).

These two initial studies confirmed that common milkweed was very sensitive to ozone air pollution within the polluted air masses being carried from long distances, such as the Midwest and Ohio River Valley, into the Blue Ridge Mountains of Virginia.

The common milkweed is considered a good bioindicator of ozone air pollution. It exhibits distinct leaf symptoms that are easy to diagnose, has few diseases, limited pest problems, and exhibits genetic stability.

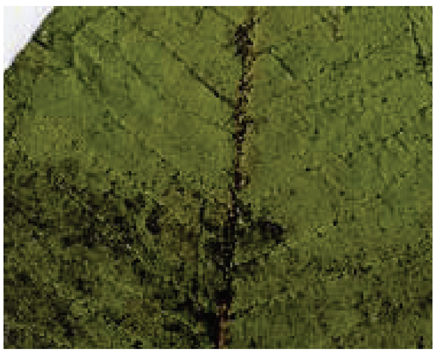
Mimicking Symptoms on Milkweed. Not all injury seen on the milkweed leaves is ozone induced. The milkweed is susceptible not only to the monarch butterfly larvae, but also to aphids, a variety of beetles, and the tussock moth. Careful observations will distinguish injury caused from ozone air pollution and symptoms caused by other stresses.

Careful assessment of these leaves show atypical brown injury that goes from the surface to the underside of the leaf. Other areas of the leaves have been eaten by insects or caterpillars.

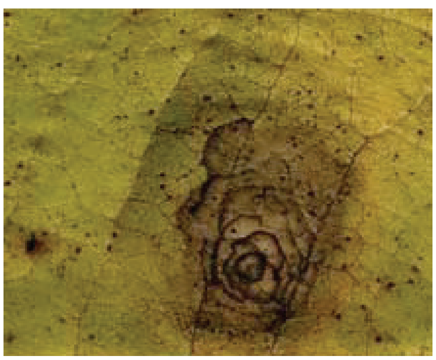
The caterpillar of the tussock moth is brightly colored and hairy. It will devour the entire leaf except for the main vein and petiole. It is common mid to late summer. It acquires chemical defenses from eating milkweed that deter predators such as bats from eating them.

The milkweed longhorn beetle is a common species on milkweed during the summer. When the milkweed plants are in bloom, the larvae bore into the stems.

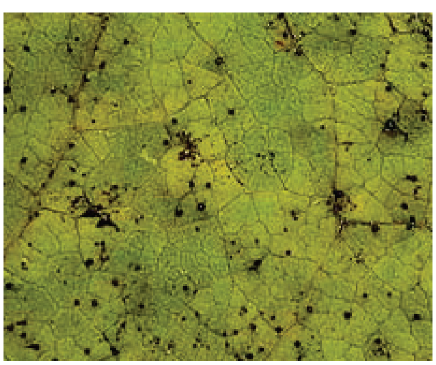
These brightly colored aphids tap into the plant and feed on its juices, but the plant injury is not obvious. The ladybug feeds on the aphids.



The surface of the leaf looks dirty from the presence of sootymold. The aphid excretions, honey dew, are left behind and colonized by a black colored fungus creating a black sooty effect. This fungus can be wiped off the leaf. There are also circular black dots on the leaf's surface that are not ozone-induced foliar injury and are visible on both the upper and lower sides of the leaf.



The dry, dead tissue on the leaf's surface is a fungus-caused injury. The injury goes from the upper through to the underside of the leaf. It will not prevent blooming of the plant or the production of milkweed seeds.



The black circular lesions on this leaf are not ozone-induced injury. The lesions have distinct edges and go through the leaf to the underside. Notice the lesions don't cross the veins, requiring a closer look to determine that this symptom is not ozone injury. Additional information may be found at EEK!—Environmental Education for Kids at www.dnr.state.wi/us/eek/.

Planting Milkweed Seeds

It is best to start seeds in small pots filled with field soil from the site where you plan to have an ozone garden with the pots placed in flats for easy watering. Fill the pots about half full, moisten the soil, place 2 to 3 seeds on top, and gently cover the seeds. Moisten the top layer of soil. As seedlings begin to emerge, leave the strongest one and cut off the weaker plants at the soil level. Pulling seedlings out of the soil may damage the fine feeder roots of the young plant you selected to subsequently plant in your ozone garden for study.

The seedlings should be carefully nurtured with watering and carefully handled. Seedlings are quite succulent and easily injured with rough handling. Fungal growth can kill the seedlings, so the soil should not be kept wet. When seedlings are about 8- to 16-cm tall (3 to 6 in.), begin acclimating them to the natural environment by placing the pots in a sheltered sunny area during the day. Do this for 3 to 4 days. Bring them in at night for the first few days, and then if warm enough, let them remain outside in the sheltered area.

While the plants are acclimating, prepare the garden to receive the seedlings. Do not fertilize the area of the garden where the milkweed seedlings will be planted. Till the soil to remove vegetation and any clumps that might interfere with the start of the new seedlings. A fine consistency in the soil will ensure a good start. Plant the seedlings 36 cm (18 in.) apart. The young seedlings will need to be watered very lightly the first 8 to 10 days in the early morning. Don't overwater, but keep the soil moist to give the seedlings time to become established in the garden. In some instances, seedlings of both common and tall milkweed may take 2 years to fully develop into thriving plants.

Planting Seedlings from Pots. The garden has been tilled and clumps and unwanted vegetation removed. If milkweed plants were started in pots, do the following to plant them:

1. Dig a hole the size of the pot.
2. Moisten the soil.
3. Turn the pot with the seedling onto its side and gently tap the pot to loosen the soil from its sides.
4. Gently remove the plant with its soil and place it in the hole matching thesurface of the potted plant with the ground's surface.
5. Gently tap down the soil and place more soil on top of the area if needed.

Planting Seeds in Tilled Soil. The soil has been tilled and weeded and now ready to receive the milkweed seeds. Poke 2 to 3 holes about 2 cm (1/2 in.) deep in a small circle using your finger. Drop a milkweed seed in each hole and cover lightly. Mist the surface of the soil if it is not moist from earlier watering. Create 3 more areas about 36 cm (18 in.) apart using the same process. When the young plants emerge, keep the strongest plant in each section and carefully cut off the extra seedlings at the ground line to allow the single plant to grow for data gathering.

In the following years of maintaining the garden, you may limit the spread of the common milkweed by removing seedpods and monitoring new growth in the spring. Clipping the newly emerging seedlings that arise in the spring from underground rhizomes (except, of course, those selected for continuing study) will assist in keeping the spread to a minimum.

Assessing Injury to Milkweed

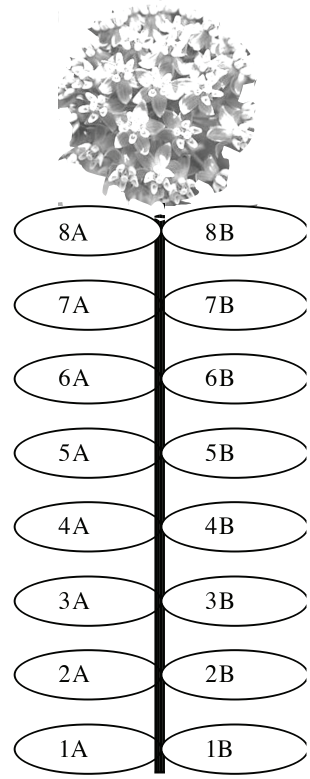
Labeling Plants and Leaves for Observation. Each milkweed plant needs to have its own ID label. It should be located on a garden stake placed at the base of the plant, but an additional large tag attached to the base of the plant will enable observers to identify the plant if the stake gets damaged or removed.

Before the milkweed leaves show ozone-induced foliar injury, collect baseline data on each plant. Use a meter stick to measure the height of each plant and record the measurement in centimeters. Carry and place a thin board on the ground at the base of each plant during measurements. This is done to provide a stable surface for the height measurements; i.e., this provides a good platform for consistent measurements by not pressing the meter stick into the ground by differing observers. If the plant is not fully grown, measure only up to the base of the top-most leaf. Count the number of leaves coming off the main stem and note flowering, nodes that have lost leaves and any other changes that appear each week.

The leaves on the milkweed plant are directly across from each other so it will be important to label the leaves as they emerge for recording ozone-induced foliar injury. The following process for each plant's ID and leaf labeling is recommended to ensure consistency and accuracy in data gathering:

- The ID for each milkweed plant begins with C for common and T for tall followed my MW for milkweed. The four plants will have the following ID sequence CMW or TMW for the specific cultivar followed by a number. The following sequence would be used to ID common milkweed: CMW1, CMW2, CMW3, and CMW4. The C would be replaced with a T in the plant ID, if using tall milkweed.

- The leaves on the milkweed plant are opposite each other. **Labeling of leaves starts from the bottom of the plant upward toward the top**. While facing north, the first leaf pair on the stem would be labeled 1A to the left and the leaf on the right of the stem would be labeled 1B. Repeat this process as new leaf pairs emerge at the top of the plant throughout the early part of the growing season. Use the same labeling process for the three other milkweed plants using one of the plant ID codes: CMW2, CMW3, and CMW4 for identification. This extra step will prevent different observers from mixing up data on the data sheet.



Careful labeling of plants will enable several people to accurately identify each plant for recording data. At the season's end, place a rod with a red flag identifying the individual plant's number. This will serve as a marker to identify the new plant for the next year.

Materials Needed for Plant Observation. A variety of materials are needed in preparation for assessing foliar injury. Organizing the needed materials in a container to consistently transport them to the area where the milkweed plants are growing will simplify the task.

1. A meter stick to measure the height of the plant; a small flat board will assist in making height measurements when placed under the meter stick for stability on the soil surface.
2. A 10x magnifying glass to initially find the stippling and to carefully examine leaves form small insects and the presence of fungus.

3. Milkweed field guide (ozone-induced leaf injury identification chart)
4. Small tags with string, and black fine point marker to label new milkweed leaves.
5. Clipboard with data sheets and pencil.
6. Digital camera for periodically recording the progression of symptoms.

Plant Assessment Field Procedures-Ozone Induced Effects. Using a 10x magnifying glass enables the observer to see the beginning of stippling. Ozone-induced foliar injury may become visible by mid-June and more likely even later in the summer season. Beware of mimicking symptoms and other pest problems that may produce symptoms that look like ozone-induced foliar injury to sensitive milkweed. One strategy is to look at older leaves, as they should have the most injury. If younger leaves have the most injury, it most likely isn't ozone induced. The stippling should be on the upper leaf surface of the leaf only and not on the underside. Also, stippling does not affect or appear on any of the leaf's veins.

Use the following steps to assess ozone-induced foliar injury and record your data on the data sheet for the milkweed species. Use a different data sheet for each plant. During each observation period:

1. Work in teams to measure the plant's height from base to apex using a meter stick and record the height in centimeters. Place the meter stick on a smallpiece of wood to keep it from depressing into the ground. Measure the plant's height from the base of the plant to the base of the petiole of the highest leaf.
2. Only the 10 bottom leaves are numbered and if, after labeling, a leaf falls off near the bottom of the plant, it is still counted as one of the original ten leaves used for data gathering. Carry forward each week the last data that was previously recorded for the fallen leaf.
3. Assessing injury to a milkweed leaf may be done by looking at the whole leaf to estimate the percentage of injury. The milkweed ozone-induced injury appears only on the upper surface of the leaf.

4. Check the plant ID and the data sheet ID to ensure that the two identifications match, before gathering data.
5. Each leaf is rated for:
   a. S = Stippling (black-purplish color leaf surface)
   b. C = Chlorosis (yellowing in the leaf indicate loss of chlorophyll in cells
   c. N = Necrosis (portion of the leaf that is dead)
6. Estimating the percentage of injury to a leaf requires practice. To practice and develop this skill use the resources found at:
   www.ozonegarden.nasa.larc.gov
   www.2nature.nps.gov/air/edu/O3Training/index.cfm.

The ratings are given along with their percentage values. For example, if the plant has no ozone-induced stipple, it would be given a rating of Class 1, whereas if it shows beginning symptoms it would be rated Class 2. A range of six classes is used to assess ozone induced foliar injury and are identified below:

Class 1 = 0%	No ozone-induced injury
Class 2 = 1 to 6%	Light ozone-induced injury
Class 3 = 7 to 25%	Moderate ozone-induced injury
Class 4 = 26 to 50%	Moderately severe ozone-induced injury
Class 5 = 51 to 75%	Severe ozone-induced injury
Class 6 = 76 to 100%	Extremely severe ozone-induced injury

1. Plants should be assessed for symptoms only on bright sunny days and with the sunlight over the observer's shoulder. Observations for symptoms should be continued at 2- to 3-day intervals, until the first symptoms of typical ozone-induced injury appear. Continue assessing the plants once a week, on the same day of week or as close as may be possible, providing there are sunny conditions.
2. Record the number of flowers and then the number of seedpods per plant as they develop near the season's end.
3. When a leaf falls off, carry its last data entry over into all of the ensuing data collection entries. This is to show leaf loss on a graph.
4. If insects eat part of a leaf or if various diseases appear, rate only the surface you can see for ozone-induced injury and identify the percentage of leaf missing.

As the exposure to ozone concentrations increases, foliar injury will increase. Also, a leaf may simply break off the stem. If the leaf is still present on the ground at the plant base, and preferably with the paper tag attached, look at the dropped leaf and assign it the appropriate leaf loss code using the following criteria:

Class 7 = leaf dropped with no prior symptoms or just missing

Class 8 = leaf dropped with prior chlorosis (yellowing) only

Class 9 = leaf dropped with prior stippling (the tan to brownish dots) only

Class 10 = leaf dropped with all or some of the following: stippling (small dark colored dots), chlorosis (yellowing), or necrosis (browning)

Filling in the Data Sheet. A data sheet has been designed for gathering the percentage of ozone-induced foliar injury on the first eight leaves on each side of the milkweed plant (total number of milkweed leaves tagged equals 16). Please note that a plant **ID number must be written at the top** of each data sheet.

The plant ID codes for four common milkweed plants are the following: CMW1 for the first plant, CMW2 for the second plant, CMW3 for the third plant and CMW4 for the fourth plant. The letter T for tall milkweed would be used in place of the C, using the following ID codes for four tall milkweed plants: TMW1, TMW2, TMW3, and TMW4.

When collecting data on the individual leaves, start at the base of the plant, with leaf #1A, and leaf #1B, and assess up to the first eight pairs of leaves for injury. Data from the eight leaves each side, on a single plant, may be recorded on each data sheet next to the identified leaf.

Milkweed Field Guide

0% Leaf Injury = Level 1

Rating System:
1 = 0% injury
2 = 1-6% injury
3 = 7-25% injury
4 = 26-50% injury
5 = 51-75% injury (leaf has very small green areas
6 = 76-100% injury (Leaf has no small green areas)

1-6% Leaf Injury = Level 2

Lower end of injury Level

Higher End of Injury Level

7-25% Leaf Injury = Level 3

Lower end of injury Level

Higher End of Injury Level

26-50% Leaf Injury = Level 4

Lower end of injury Level | **Higher End of Injury Level**

51-75% Leaf Injury = Level 5

Lower end of injury Level | **Higher End of Injury Level**

76-100% Leaf Injury = Level 6

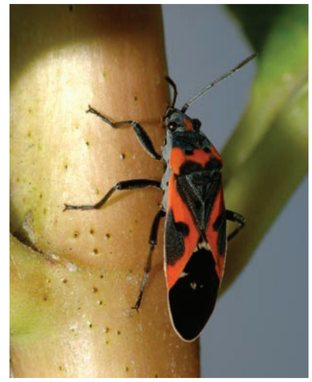
Lower end of injury Level | **Higher End of Injury Level**

Milkweed Injury NOT Ozone Induced

Note: Ozone-induced injury <u>never</u> has light colored holes with rings or leaves with a substance that can be rubbed or washed off. Stipple appears only on the upper leaf surface and NOT the underside of the leaf.

Injury caused by larvae of monarch butterfly

Injury caused by the caterpillar of tussock moth MonarchWatch.org

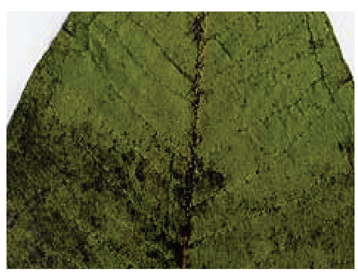


Milkweed beetle S. Ellis, Bugwood.org

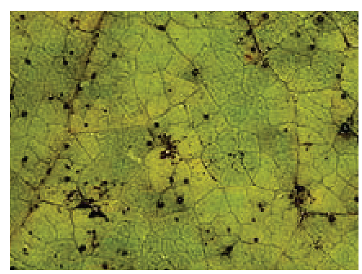
Milkweed longhorn beetle P. Sloderbeck, Kansas State University. PDIS

Aphids on milkweed MonarchWatch.org

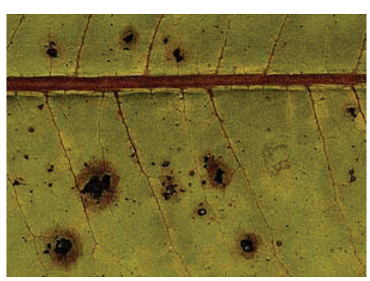
Dark brown injury on the lower left side is not ozone-induced, and goes from the upper to the lower side of the leaf



Black circular lesions E. Jepsen, Wisconsin Deptartment of Natural Resources



Fungal disease on leaf E. Jenson, Wisconson Department of Natural Resources



Light colored ringsaround necrotic areas mimic ozone-induced injury E. Jepsen, Wisconsin Deptartment of Natural Resources

Aphid honey dew turns blackish in color by fungus growth on the leaf surface and can be rubbed off E. Jepsen, Wisconsin Deptartment of Natural Resources

Leaf A8 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf A7 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf A6 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf A5 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf A4 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf A3 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf A2 | Leaf present ☐ | Leaf Missing ☐

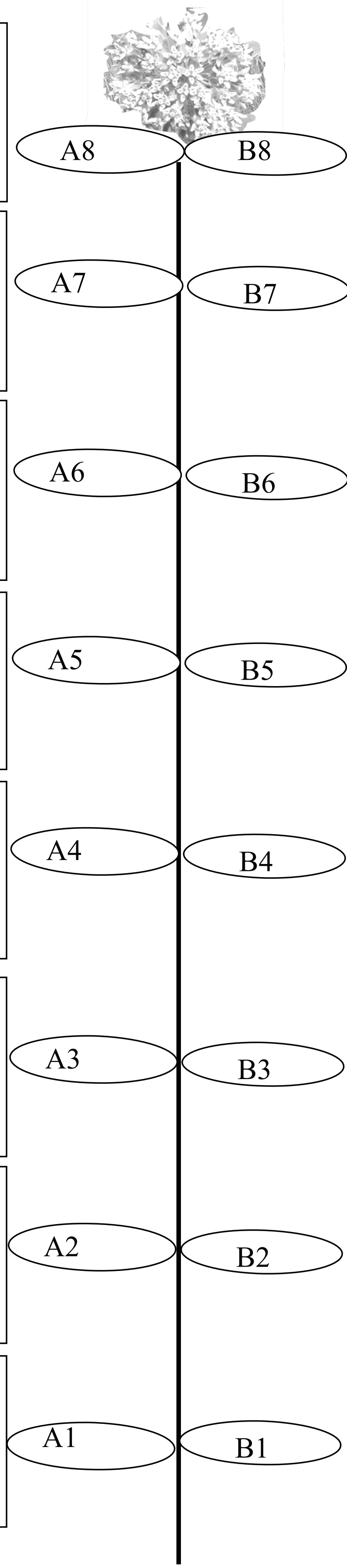
	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf A1 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:



Leaf B8 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf B7 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf B6 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf B5 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf B4 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf B3 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf B2 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Leaf B1 | Leaf present ☐ | Leaf Missing ☐

	0%	1-6%	7-25%	26-50%	51-75%	75-100%
Stipple						
Chlorosis						
Necrosis						

Observations:

Appendices

Field Guide

Site Definition Sheet

Group/Club/School Name:_______________________________ Date:_____________

Participants filling in site definition sheet: _______________________________________

____

A site definition sheet identifies the location of your site and the plants included in the ozone bioindicator garden.

Location: Latitude _______________ Longitude______________

Elevation:______________________meters

Source of Location (check one) ____GPS _____Other (please specify)______________

Plants in garden (check all that apply) ____snap beans ____cutleaf coneflower ____common milkweed ____tall milkweed

Describe site (include trees, buildings, etc., that are near your site) ____________________

____

____

Draw a model of your garden layout. Label north, south, east and west and place markers in the garden to identify **N S, E, and W**. Identify the **length and width** of your garden in meters and centimeters. (Sample garden layouts are located in the ozone bioindicator guide) Draw a layout of your garden in the box.

Width in meters/centimeters

Go to: ozonegarden.larc.nasa.gov/ and register your site. If possible, include a photo of your garden as it was being planted, and later a photo showing the plants growing and being labeled and data being gathered.

Field Guide
Assessing Ozone-Induced Foliar Injury

Materials

- Meter stick
- Tags with string, and black fine point permanent markers
- Site location flag for each plant with the plant number on the flag
- Plastic tag with plant ID number to be tied at the base of plant
- percentage ozone leaf injury identification chart for each plant species being assessed
- Clipboard and pencil
- A 10x magnifying glass to initially find the stippling and carefully examine leaves for small insects and presence of fungus
- A compass to identify north, south, east, and west
- GPS to identify latitude and longitude of site
- Thin piece of wood to place under the meter stick to prevent it from depressing
- into the ground when measuring plant height
- A tote bag to carry all field materials

Note: The first evaluations should occur at least by June 15th. The final evaluation should be before the first hard frost in your area.

Procedures for Plot Evaluation

1. Use a 10x magnifying glass to check leaves daily until you see the beginning of stippling, and record observations. Ozone-induced foliar injury may become visible mid-June through the end of the summer season depending upon the geographic location of the site.
2. Plants should be assessed for symptoms only on bright sunny days and with the sunlight over the observers shoulder. Observations for symptoms should be continued at 2- to 3-day intervals, until the first symptoms of typical ozone-induced injury appear. Continue assessing the plants once a week, on the same day of week or as close as may be possible, providing there are sunny conditions.
3. Measure the individual height of each plant from base to apex, using a meter stick and record the height in centimeters. Always place a thin piece of wood under the base of the meter stick to prevent it from depressing into the ground.

4. Measure the plant from the bottom to the base of the petiole of the most open leaf. Once the plant is in bud or flower, hold the plant straight and measure to the top of the tallest bloom.
5. Count the total number of leaves from the base to the top of the plant. **Do not count** leaves that are not fully expanded. (In general, evaluate only leaves that are at least 65% expanded) **Do count** leaf scars where leaves have fallen off (run your hand up the stem to feel for leaf scars).
6. The ratings are given along with their percentage values. For example, if the plant has no ozone-induced foliar injury, it would be given a rating of Class 1, whereas if it shows a few symptoms a rating of Class 2. The range of six rating classes used to assess ozone induced foliar injury are identified below:

Class 1 = 0%	No ozone-induced injury
Class 2 = 1 to 6%	Light ozone-induced injury
Class 3 = 7 to 25%	Moderate ozone-induced injury
Class 4 = 26 to 50%	Moderately severe ozone-induced injury
Class 5 = 51 to 75%	Severe ozone-induced injury
Class 6 = 76 to 100%	Extremely severe ozone-induced injury

7. If a fallen leaf is available, record its loss and then carry the previous data through on each subsequent data sheet. This is to show leaf loss on a graph as opposed to leaf improvement. Assign it the appropriate leaf loss code:
   a. Class = 7 Leaf gone with no prior symptoms or leaf gone no data
   b. Class = 8 Leaf gone with prior chlorosis (yellowing) only
   c. Class = 9 Leaf gone with prior stippling only (discrete purple spots)
   d. Class = 10 Leaf gone with prior stippling, chlorosis, and necrosis

8. Be very careful when checking the underside of the leaf. They are fragile and will break off easily.
9. Never make assumptions about what you are seeing, just rate what you see at that point in time. If unsure what the symptoms are, e-mail a photo and your question to: http://ozonegarden.larc.nasa.gov for a review and response.
10. Insects will eat part of some leaves. Rate the leaf surface area that is visible, but note in the comment area the percentage of total leaf area missing.
11. Plants should be assessed for symptoms only on bright sunny days and with the sunlight over the observer's shoulder. Observations for symptoms should be continued at 2-3 day intervals, until the first symptoms of typical ozone-induced injury appear. Continue assessing the plants once a week, on the same day of week or as close as may be possible, providing there are sunny conditions. Assessments may be made until the first frost in the fall of the year.

12. **BEWARE of MIMICKING SYMPTOMS** and other pest problems that may appear to be ozone-induced injury. Some points to consider are:

   a. Look at older leaves on the plant as they should have the most injury. If younger leaves have the most injury, it is not usually ozone induced.
   b. The stippling should be <u>only</u> on the surface of the leaf and <u>not</u> on the under side of the leaf.
   c. The stippling <u>does not</u> appear on any of the veins.
   d. Carefully analyze the injury and compare your observations to the field guide photographs of ozone- induced foliar injury and photographs of injury that are caused by insects, pathogens, and other sources.

Activity: Making and Using Schönbein Paper

I.H.Ladd

Background Information

Christian Friedrich Schönbein was a professor at the University of Basel, Switzerland. He discovered ozone in 1839 and developed the following method of measuring ozone in the troposphere by mixing potassium iodide, distilled water, and cornstarch; putting the mixture on filter paper; and exposing the treated paper to ambient air for eight hours. The Schönbein paper changes color when ozone is present.

Ozone is very reactive and oxidizes any surface it contacts. For example, when it comes in contact with lungs it begins to destroy the elasticity of the lung tissue, and over time, can cause lung damage. When plants take in air, ozone enters the plant through the stomata of the leaf causing cell damage within the leaf. So, when ozone in the atmosphere comes in contact with the Schönbein paper, it oxidizes the potassium iodide on the test paper and produces iodine. The iodine reacts with the starch in the cornflower and stains the paper a shade of purple. The intensity of the purple color depends on the level of ozone present. The higher the level of ozone, the darker the response will be on the strip.

The following Web sites will provide more background information. If you do a computer search for *ozone air pollution,* participants can learn more about the impact of ozone on humans and plants.

NASA satellite research on Atmosphere-ozone:
http://earthobservatory.nasa.gov/Topics/atmosphere.html
Information on Plants: http://www.ars.usda.gov/Main/docs.htm?docid=8453
Ozone –ozone now, ozone alert days, and more : http://www.airnow.gov/
Children and air pollution: http://www.lungusa.org/site/pp.asp?c=dvLUK9O0E&b=44567
Curriculum integration ideas: www.globe.gov
Resource for Schönbein Project: http://www.ucar.edu/learn/1_5_1.htm,
http://www.ucar.edu/learn/1_7_2_29t.htm

Age Level/Time

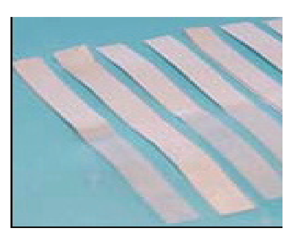
- **From age:** 14 upward
- **Time to produce strip:** will vary depending on how quickly students work
- **Exposure time:** 8 hours

Materials

- Potassium iodide granular (a teaspoon will be plenty and it can be put in a test tube to give to students)
- Corn starch (can also be put in glass test tube for each student team)
- 100 ml beaker
- 250 ml beaker
- Plastic measuring spoons
- One plastic spray bottle per team
- Gallon of distilled water- a must
- Whatman filter paper Grade No. 2
- Glass stirring rod (do not use metal)
- Small paint brush
- Glass baking plate
- Hot mitt for handling the hot beaker
- Heat source (preferably a hot plate- boil water and provide each team 100 ml to pour into 250 ml Beaker)
- Scissors
- Student data and Schoenbein analysis sheets (one each team)

Procedure to Make Strips

1. Place 100 ml of distilled water into a 250 ml beaker.
2. Add ¼ teaspoon of corn starch.
3. Heat and stir mixture until it gels. The mixture is gelled when it thickens and becomes somewhat translucent.
4. Remove the beaker from the heat source and add ¼ teaspoon of potassium iodide and stir well.
5. Cool the solution before applying it to the filter paper.
6. Lay the filter paper on a glass plate (or you can hold it in the air) and carefully brush the paste onto one side the filter paper. Do the same thing to the opposite side. Apply paste uniformly.
7. Attach the untreated section of the paper to a clip, out of direct sunlight, so it can dry. A drying oven works well if one is available.
8. Wash your hands after applying the potassium iodide mixture. The potassium iodide is not toxic, but can cause a mild skin irritation.
9. When paper is dry, cut it into 1-in. wide strips.
10. Store the strips in a sealable plastic bag or glass jar out of direct sunlight.



Procedure to Test Strips

Determine where you want to place strips. The site **must be out of direct sunlight.** Inform the students that their strip(s) must hang freely to allow ambient air to flow freely around their strip(s), and it must be exposed for 8 hours.

Need:

1. DATA SHEET-Give each student a data sheet to record:
   a. Site location-record on non-treated part of strip
   b. Date- record at top of non-treated part of strip
   c. Time strip is placed
   d. Time strip is read
   e. ppb (parts per billion)
   f. Relative humidity
2. At least 2 two test strips to test
3. Spray bottle with distilled water

Testing Procedure

1. Spray each test strip with distilled water.
2. Hang the strip at the identified site. Be sure it is out of direct sunlight and hangs freely.
3. Record the site where each strip is located (if you use sites located at student homes or different areas of your community, mark a local map so students can see where different levels of ozone were measured).
4. Place a digital hygrometer at each site location.
5. Expose the strip for 8 hours.
6. After exposure, place the strip in a zip lock bag and return to the classroom to analyze the strip.
7. To observe and record test results, spray the **exposed** strip with distilled water. Observe the color.

NOTE: High humidity does affect results, so the Schönbein paper should not be left outside during periods of high humidity. Participants are welcome to experiment during periods of low and high humidity to observe how it effects the strip.

Observations and Questions

Q. Why isn't the color on the strip uniform?

A. It may be due to the consistency of brushing on the chemical mixture. It may depend upon the amount of oxidation.

Q. When students compare their exposed strips they may find a variation in the levels of color change, why?

A. The responses of the test strip will vary depending upon where students place the strips. For example, one placed in the copy room near the copying machine may show more color than one in the classroom. The copy machine produces ozone. Another example, sites near highways may show a greater color change due to the oxidants from the vehicles and nitrous oxides from heavy nearby traffic.

Q. How does high humidity affect the test strip?

A. Water is a reactant, so high humidity will affect how the Schönbein strip reacts. Sites near lakes or streams may have greater color change.

Q If students placed a strip at home and one at a different site, why might the color change of the two strips vary? Or why might the color change be different at different sites?

A. Ask the students what they think? Concentrations of ground level ozone vary depending on the amount of traffic in the area, wind direction, and other climate conditions. They may want to record cloud cover and type, temperature, weather, and humidity to determine if there are any correlations.

Q. After students discuss their results and problems they may have had with their data collecting, ask them: Describe whether you think this method is a good way to measure ozone in the troposphere.

A. One difficulty the students will identify is the difficulty they had interpreting the color accurately. They will also note the difference in the consistency in producing the strips, however, stress that it is a good method for measuring *relative amounts of ozone*.

Q. How close is the 8 hour measurement using Schönbein test strips to the 8-hour measurements taken by the local Air Quality Control Center?

A. Unless their strip is placed next to the AQC site, their measurements may vary. Get local readings and see if student readings agree with or are near the range measured by the ozone-monitoring instrument.

Q. Does ozone vary each day at the same location?

A. Have the students gather data at their specific site(s) for several days for. Plot that data on the *Schönbein Humidity Graph*.

Assessing Learning

Preparing student and community members for an out of school inquiry activity may necessitate providing training on writing up the lab and a report on the findings. Methods of presenting findings may include: a power point presentation, written report, artistic display, oral presentation, or other options determined by the participants may be considered as part of assessing this activity.

Guide to Measuring Ground Level Ozone
Using the *Schönbein* Strips

- You will need the *Schönbein Test Strips* you exposed for 8 hours and the humidity measurement recorded by a digital hygrometer placed at the site.
- When you collect your exposed strip, record the relative humidity.

Schönbein Color Change Chart
(After 8 hours of exposure)

The above chart is an estimate based on the Schönbein Number and the description of color change.

Schönbein Number	Level of color change
1-3	Little or no change
1-6	Lavender hue
7-10	Blue or purple

- Use the *Schönbein Color Change Chart* and determine the *Schönbein number* of your exposed test strips. As you can see from the color scale, the darker the color, the higher the number. Work with your team to determine the *Schönbein* number, and be sure everyone on the team agrees.

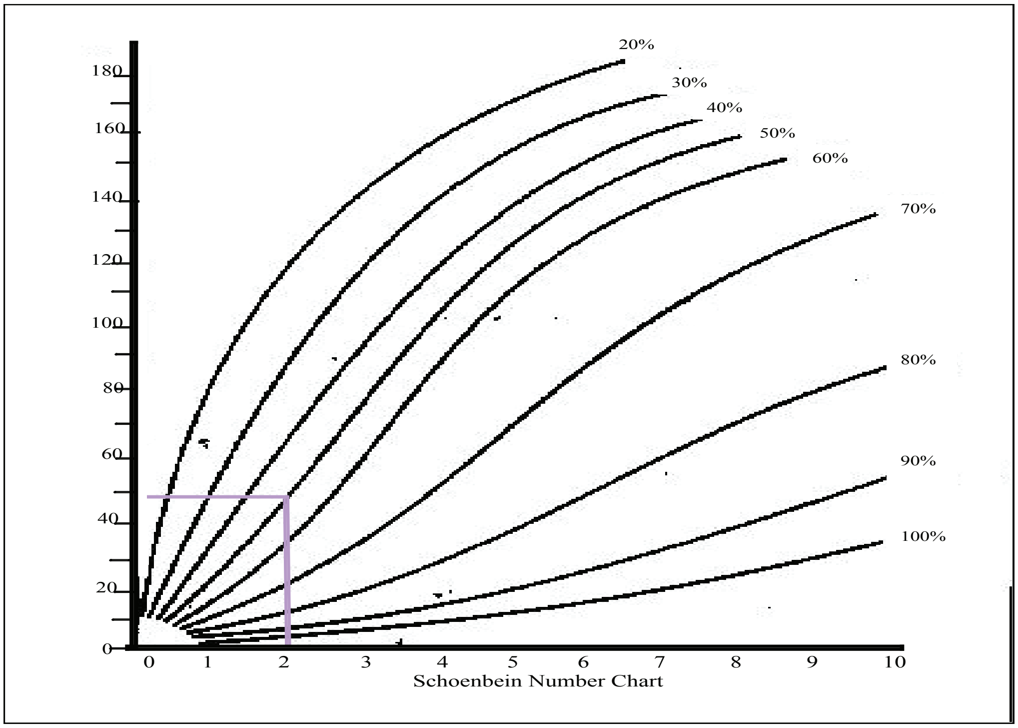
Record the *Schönbein Number* for each test strip as shown in the sample in table 1.

Table 1: Sample: Data from Schoenbein Strip

Date	Time Place	Time Read	Location	Schönbein Number	Relative Humidity	Ozone (ppb)	Ozone Monitor ppb
6/7/007	8 a.m.	4p.m.	Site 1 Teacher Parking Lot	2	50%	45	

- Use the Schönbein Humidity Chart, find the Schönbein number for your exposed strip on the X-axis, and draw a straight line up to the curve that matches the relative humidity you measured.
- From the point that your straight line touches the curved humidity line, draw another line across to the Y-axis. The point at which this new line crosses the Y-axis is the ozone concentrations in ppb (see example in table 2).

Table 2: Sample *Schönbein Humidity Chart* with Plotting Sample- color change 2 at 50-percent relative humidity. The ozone measurement falls just below the 50 ppb. The team should work together and agree on the number from the color chart and the final level of ozone.



1. What factors affect the reliability of the Schönbein Test Strips?

2. What limitations exist in using the Schönbein paper to measure ozone?

3. What can students learn from using Schönbein Test Strips?

Sample of Schönbein Data and Plotting Results on a Graph

For Data from Schoenbein Strip - by I.H. Ladd

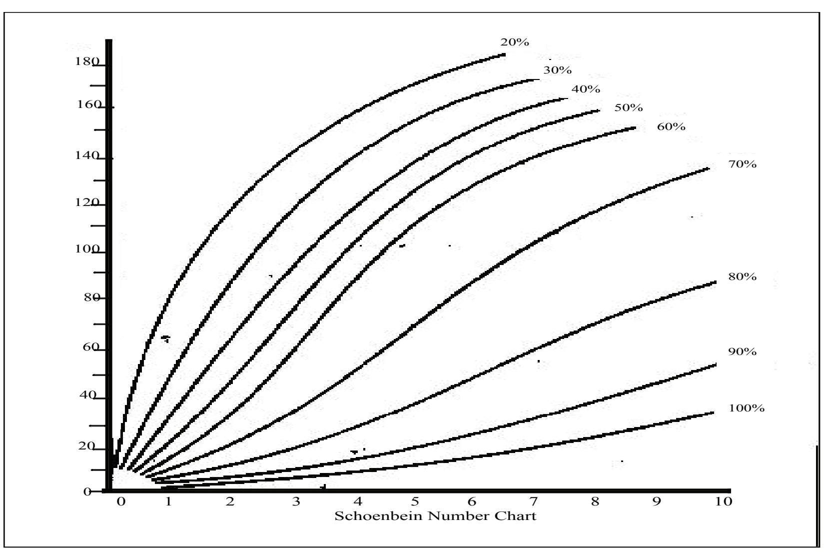
Date	Time Place	Time Read	Location	Schönbein Number	Relative Humidity	Ozone, ppb	Ozone Monitor, ppb
6/7/007	8 a.m.	4p.m.	Site 1 Teacher Parking Lot	2	50%	45	

Schönbein Color Change Chart
(After 8 hours of exposure)

The above chart is an estimate based on the Schönbein Number and the description of color change.

Schönbein Number	Level of Color Change
1-4	Little or no change
1-7	Lavender Hue
7-10	Blue or Purple

Pick the Schönbein number for the color closest to the color response on your chemical strip. Use the Schönbein Humidity Chart in your Field Guide for GROUND LEVEL OZONE TESTING and find the X-axis. Draw a straight line up to the curve that matches the relative humidity you measure. Sample below.



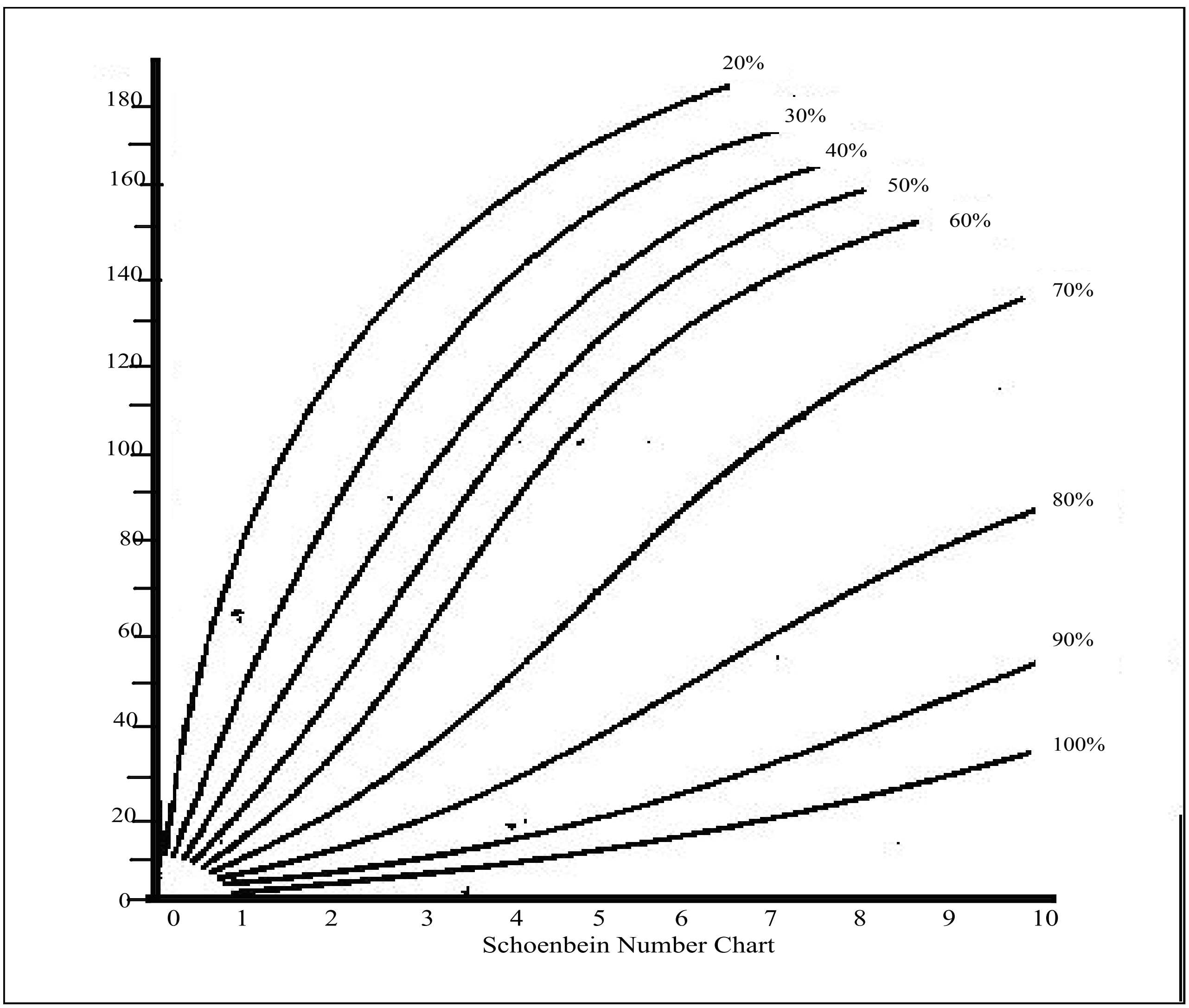
Team: Date:

FIELD SHEET – Schönbein Plotting Chart

Adapted by Irene Ladd from: http://teachertech.rice.edu/Participants/lee/colorscale.html

0 3 4 6 7 10

Schoenbein Number on Color Scale	Level of Color Change
0-3	Little or no change
4-6	Lavender Hue
7-10	Blue or Purple

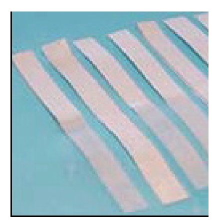


Lab

Making and Testing Schönbein Paper

Materials

- ¼ teaspoon iodide granular
- 100 ml beaker
- 250 ml beaker
- set of plastic measuring spoons
- Spray bottle with distilled water
- Whatman filter paper Grade No. 2 (2 filters)
- 1 ¼ teaspoons of cornstarch
- glass stirring rod
- small paint brush
- glass Pyrex plate
- hot mitt
- paper clips to hang treated paper on line to dry and string
- hot plate (If not one each team, then one member with mitt get 100 ml of boiling water that has cornstarch mixed into it, and pour into 250 ml beaker to use for mixing
- procedure)
- Scissors



Procedure to Make Schönbein Paper

1. Place 100 ml of distilled boiling water into a 250 mL beaker.
2. Add 1 ¼ teaspoon of corn starch.
3. Heat and stir mixture until it thickens and becomes somewhat translucent. Add cornstarch when water is cool and stir constantly. (This step may have to be done at the central area where the hot plate is located.)
4. Remove beaker from heat source.
5. Add ¼ teaspoon of potassium iodide and stir well using the glass rod.
6. Lay the filter paper on a glass plate, or hold in the air, and carefully brush the paste onto the filter paper.
7. Turn filter paper over and do same to opposite side (try to apply paste uniformly as possible).
8. Hang filter paper to dry out of direct sunlight.

9. Wash hands after applying the potassium iodide mixture. Potassium iodide is *not toxic*; however; it can cause a mild skin irritation.
10. Once the filter paper is dry, cut it into 1-in. wide strips.
11. Store the paper in a sealable plastic bag or glass jar out of direct sunlight.

Field Guide

Exposing and Reading the Schönbein Strips

Materials for Field Work

- Clipboard with data sheet and pencil
- Two or more Schönbein Strips
- Spray bottle with **distilled water**
- Schönbein Chart for strip analysis
- Digital hygrometer (follow Globe Protocol for using digital hygrometer)

Testing Procedure

1. Fill in data sheet information at site:
   a. Team
   b. Date
   c. Time placed strip (write location on top of untreated part of strip)
   d. Location of strip (write location on top of untreated part of strip)
2. Spray a test paper with distilled water
3. Hang the strip at site out of direct sunlight
4. Expose the strip for 8 hours
5. When you return to get exposed strip, seal it in a zip lock bag
6. Record relative humidity
7. Return to classroom to read the strip

Reading the Exposed Strip

1. Remove exposed strip from zip lock bag
2. Spray strip of test paper with distilled water
3. Compare the color changes on the Schönbein strip to the Schönbein color chart and select the number of the color scale that is closest to the color on the exposed Schönbein strip
4. Team agrees on Schönbein color number
5. Find the Schönbein number on the X-axis on the Schönbein humidity chart
6. Draw a line from the Schönbein number to the line representing the relative humidity
7. Draw a line from the relative humidity line to the ppb line
8. Record the ppb on your chart

Team:__________________________ Date:____________________

FIELD SHEET- *Schönbein* Data Table

Step 1: Using the Schoenbein Color Scale to Identify the Number of the color change of the exposed Schoenbein Strip.

0 3 4 6 7 10

Schoenbein Number on Color Scale	Level of Color Change
0-3	Little or no change
4-6	Lavender Hue
7-10	Blue or Purple

Pick the Schoenbein number for the color closest to the color response on your chemical strip. Work with your team to determine the final number.

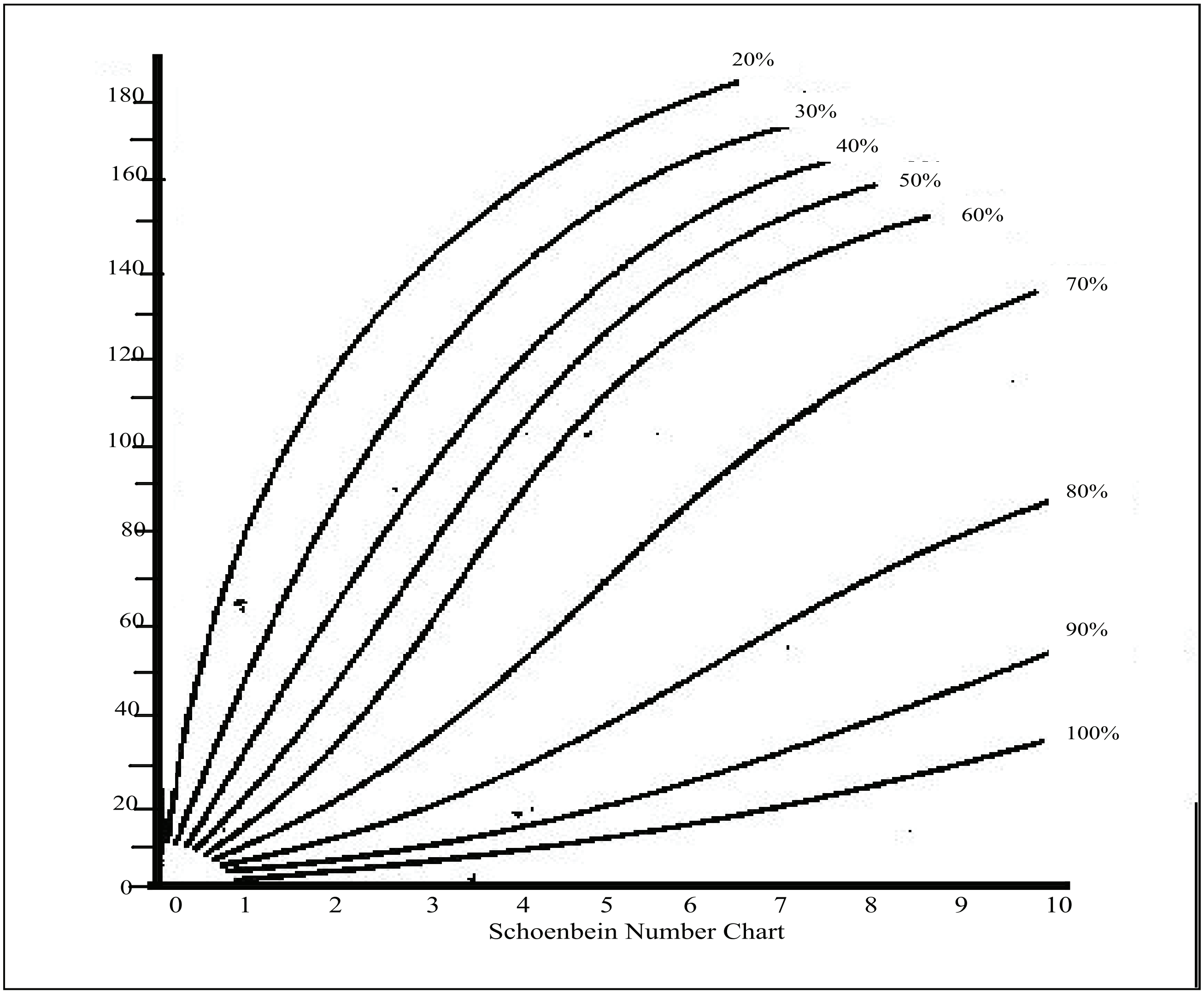
Team:__________________________ Date:_______________

FIELD SHEET- *Schönbein* Graph for Plotting Data

STEP 2: Plotting the Data from the Exposed Schönbein Strip. The strip must have been exposed 8 hours.

Use the ***Schönbein Humidity Graph*** to PLOT the LEVEL OZONE TESTING. Find the ***Schönbein*** number on the X-axis. Draw a straight line up to the curved line that matches the relative humidity you measured. Draw a second line from the line marking the humidity level to the Y-axis. This will identify the level of ozone air pollution present. All final plotting to be agreed upon by your team.

Schoenbein Humidity Graph



Electronic Resources

NASA's Earth Observatory

Full-length articles based upon research.

Satellite tracking.
http://science.nasa.gov/Realtime/jTrack/eos.html

Biosphere.
http://earthobservatory.nasa.gov/Observatory/Datasets/Help/bios.seawifs_anim_help.html

The ozone we breathe: effects on human health and on plants.
http://earthobservatory.nasa.gov/Library/OzoneWeBreathe/printall.php

Aura mission dedicated to health of Earth's atmosphere. Also, processes controlling air quality and a look at how Earth's climate is changing.
http://earthobservatory.nasa.gov/Library/Aura/printall.php

Nature's contribution to pollution.
http://earthobservatory.nasa.gov/Study/ContributionPollution/

Research and education partnerships.
http://earthobservatory.nasa.gov/Study/Partnerships/printall.php

Tracking tropospheric ozone, how modeling plays a key role, and the next steps in tracking ozone.
http://earthobservatory.nasa.gov/Study/GlobalTraveler/printall.php

IDEA: Infusing Satellite Data into Environmental Air Quality Applications.
http://earthobservatory.nasa.gov/Study/IDEA/printall.php

Watching ozone weather, ozone in the troposphere, ozone pollution standards, monitoring ozone, and solving the problem.
http://earthobservatory.nasa.gov/Library/OzoneWx/Ozone/printall.php

NASA's Earth Observing System

Satellite system resources such as lithographs, brochures, and pamphlets.
http://eospso.gsfc.nasa.gov/eos_homepage/for_educators/index.php

Science Serving Society series of publications.
http://eospso.gsfc.nasa.gov/eos_homepage/for_educators/educational_publications.php

Plant Information

A link to a foliar injury assessment module. A training tool used for individuals in the assessment of air pollution injury to vegetation.
www.2nature.nps.gov/air/edu/O3Training/index.cfm
www.ozonegarden.larc.nasa.gov

Information about common milkweed and ideas for a study; good source of imagery.
http://envirolink.org/external.html?www=http%3A//homepage.mac.com/cohora/nat/mon.html&itemid=200408260740500.122226

The Plant Diagnostic Information System offers an image library database users can search to assess symptoms or submit photographs. As part of the National Plant Diagnostic Network, links to U.S. regional diagnostic sites are available.
http://www.pdis.org/

Cornell University Vegetable MD Online. Plant diseases are identified by crops and images of different plant diseases are available. New plant disease alerts are posted on the site. An individual may submit a picture and question to the researchers in the field of Plant Pathology, and receive an accurate plant diagnosis and professional control recommendations. http://vegetablemdonline.ppath.cornell.edu/PhotoPages/Contributors.htm

The Center for Invasive Species and Ecosystems Health provides a variety of publications and images about plant health. Enter IPM images and go to plant images to identify plant injury by specific crop and insect images for images of insects by name.
www.bugwood.org

Hands on the Land is a national network of field classroom connecting students, teachers, and parents to their public lands and waterways. Browse the environmental monitoring programs offered at the Web site by clicking on Hands-on Science.
www.handsontheland.org

Pennsylvania State University, Department of Plant Pathology, Department of Horticulture, and Penn State Institutes of the Environment and Energy, University Park, PA, USA. For users of this site, data are archived and seasonally current ozone exposures and video cameras within open-top chambers show symptom progression on ozone-sensitive species; real-time views of distant Mount Nittany are also accessible.
http://www.arboretum.psu.edu/research/air.html

U.S. Department of Agriculture (USDA) Web site provides a plant profile on milkweed with images and distribution maps.
http://plants.usda.gov/java/profile?symbol=ASCLE

The U.S. Department of Agriculture Forest Service (USDAFS) provides air quality images of national forests and an historical gallery. Click on real time images to access different images and videos of different sites across the United States.
http://www.fsvisimages.com/

U.S. Department of Agriculture-Forest Service, Forest Health Monitoring Program. This site offers a detailed description of a real-life and on-going survey of ozone-sensitive bioindicator plants across much of the United States as a part of an extensive forest health and productivity evaluation process. Methods for determining the amount of ozone-induced foliar injury have also been included along with symptom descriptions for several common bioindicators.
http://fia.fs.us/library/field-guides-methods-proc/

U.S. Department of Interior (USDOI) Identifies ozone sensitive plant species state by state in national parks and identifies ozone sensitive plant species state by state in National Park Service Class I areas (wilderness areas in each state).
http://www2.nature.nps.gov/air/Pubs/pdf/flag/NPSozonesensppFLAG06.pdf
http://www2.nature.nps.gov/air/Permits/flag/flagDoc/app3a.cfm

Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland. Users can find descriptions of ozone-induced injuries on many species that are also native to the United States. Click on "search" and follow the instructions provided to select species, country, region, and type of symptom. Color photos of symptoms appear for many species.
http://www.ozone.wsl.ch

Air Pollution Injury on Potatoes.
http://www.omafra.gov.on.ca/english/crops/facts/91-015.htm

Wisconsin has schools participating in a milkweed survey. This site offers teacher plans, data sheets, and how to get involved with the project. Click on the slide show of ozone damage on milkweed in the column on the left side of the page. Scroll on each page and continue to click on begin the slide show or next slide. There is also a place to click on to get the list of slides and causes of milkweed injury.
http://www.dnr.state.wi.us/org/caer/ce/eek/earth/field/milkweed/index.htm

EEK! Environmental Education for Kids is sponsored by Wisconsin Department of Natural Resources. The section on milkweed provides images and descriptions of injury to milkweed.
http://www.dnr.state.wi.us/eek/

"Howstuffworks" introduces ozone pollution and how it works and affects health and plants. Very readable for children.
http://science.howstuffworks.com/ozone-pollution.htm

Ozone Air Pollution

New NASA/CSA monitor provides global air pollution view from space. There is a place to click on for animation to track clouds of air pollution as they travel across the Earth. This site provides a tool for identifying and quantifying pollution sources on a global scale.
http://www.gsfc.nasa.gov/gsfc/earth/terra/co.htm

NASA Aura satellite shows or exhibits streams of tropospheric ozone crossing the oceans and explains how tropospheric ozone is computed from the Aura satellite. This site provides science background information.
http://aura.gsfc.nasa.gov/science/top10_omi-mls-maps.html

NASA's Tropospheric Emission Spectrometer (TES) has a gallery with pictures, animations, movies, images and ozone maps that can be downloaded to provide background on NASA's satellite research.
http://tes.jpl.nasa.gov/gallery/index.cfm

Graphing Stratospheric Ozone Using Total Ozone Mapping Spectrometer (TOMS) Data.
http://www.exploratorium.edu/learning_studio/ozone/graphing.html

NOAA mapping of 1- and 8-hour average ozone and can loop through day to see transport of ozone, access temperature and weather information along with satellite images of weather conditions.
http://www.weather.gov/aq/

Real time current air quality images of condition of USDA-Forest Service monitoring locations throughout the United States. Web-based cameras updated every 15 to 60 minutes. Near real-time air quality data and meteorological data are also provided.
http://www.fsvisimages.com/all.html

National overview of air quality and ozone air pollution levels by good, moderate, etc.
http://www.airnow.gov/

The National Climate Data Center is the official site to get certified climate data. From this site online data can be selected, climate maps, Global Surface Data and other information is available.
http://www.ncdc.noaa.gov/oa/dataaccesstools.html

HAZECAM provides real time air pollution and visibility monitors for air pollution. This site has real time photo and visibility monitoring and provides photos of sites that can be downloaded. Select from the menu across the top of the page.
http://hazecam.net

A link to various publications related to air quality available through the National Park Service.
http://www2.nature.nps.gov/air/pubs/ozone.cfm

This Web site provides some information and maps regarding environmental health and toxicology. Enter ozone and a zip code to access specific areas or click an area of the map provided.
http://toxmap.nlm.nih.gov/toxmap/main/index.jsp.

Students can explore and learn about the ozone problem in troposphere. They can use data sets to produce graphs comparing monthly and yearly ozone averages of several U.S. Cities. The home page offers data access, lesson plans and computer tools across the top of the site.
http://mynasadata.larc.nasa.gov/L9_Murphy.html

The History of Ozone. The Schönbein Period, 1839-1868
Mordecai B. Rubin, Technion-Israel Institute of Technology
http://www.scs.uiuc.edu/~mainzv/HIST/awards/OPA%20Papers/2001-Rubin.pdf

Schönbein color charts.
http://www.chemistryland.com/CHM107Lab/Lab4/DetectOzone/Lab4Ozone.htm

Relative Humidity Schönbein Number Chart.
http://njnie.dl.stevens-tech.edu/curriculum/norwich/schoenbeinpaper.shtml

“Measuring Ground-Level Ozone” ChemMatters, September 2002, p. 8-9. “Making and Using Schönbein Paper.”
http://portal.acs.org:80/portal/fileFetch/C/CTP_005388/pdf/CTP_005388.pdf

University Corporation for Atmospheric Research Project Learn Atmospheric Science Explorers. Provides background information and educational goals for using Schönbein paper.
http://www.ucar.edu/learn/1_7_2_29t.htm

Additional Web Sites

NASA Langley Research Center’s Educational Outreach Links: MY NASA DATA, S’COOL, a glossary, an extensive links listing, and more.
http://science-edu.larc.nasa.gov/

NASA’s Agency-wide Educator Resources Search Portal.
http://search.nasa.gov/search/edFilterSearch.jsp?empty=true

"How stuff works" introduces ozone pollution and how it works and effects health and plants. The site is very readable for students.
http://science.howstuffworks.com/ozone-pollution.htm

Simulations where one can control elements that influence the development of ozone air pollution. This site provides a self-guided tour identifying what you will need for equipment and what to do to operate the program.
http://www.smogcity.com/
http://www.smogcity2.com

Digital Library for Earth System Education.
http://www.dlese.org/library/index.jsp

Atmospheric Sciences Competency Activities.
http://asd-www.larc.nasa.gov/edu_act/edu_act.html

Atmospheric circulation.
http://pubs.usgs.gov/gip/deserts/atmosphere/

National Science and Mathematics Standards Web Sites

Table of contents for the National Science Standards providing access to an overview, goals, specific content standards, professional development for teachers and assessment standards.
http://www.nap.edu/readingroom/books/nses/

National Science Content Standards for Grades 5-8. Each standard is supported with information on: student understandings, a guide to the understanding the standard and the basic concepts and principles of each standard.
http://www.nap.edu/readingroom/books/nses/6d.html#csa58

National Science Content Standards for Grades 9-12. Each standard is supported with information on: student understandings, a guide to the understanding the standard and the basic concepts and principles of each standard.
http://www.nap.edu/readingroom/books/nses/6e.html#csa912

The National Council of Teachers of Mathematics Web site provides the principles and standards for mathematical education. One must be a member to access grade level specific content standards.
http://standards.nctm.org/

Provides a general list of The National Mathematics Standards, but to access content specific standards one must be a member of the National Mathematics Teacher Association or purchase the book.
http://cnets.iste.org/currstands/cstands-m.html

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Contributors

Dr. Kent Burkey is a research plant physiologist with the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) and an associate professor of crop science at North Carolina State University. He received an undergraduate degree in physical science from Warren Wilson College in 1977 and a Ph.D. in biochemistry from Ohio State University in 1981. Dr. Burkey joined the USDA-ARS in 1983 to conduct research on photosynthesis and factors regulating chloroplast function. His research priorities have since shifted to the current research on plant response to ozone stress and identification of ozone tolerance mechanisms through studies of ozone-sensitive and tolerant plants.

Dr. Art Chappelka is a professor in the School of Forestry and Wildlife Sciences at Auburn University, Auburn, Alabama. He received his Ph.D. in plant pathology from Virginia Polytechnic Institute and State University in 1986. Since 1987, Dr. Chappelka has been investigating the responses of forest trees and associated plant species to air pollutants. Dr. Chappelka has authored or co-authored over 50 peer-reviewed journal articles and 12 book chapters. He is a reviewer for several international journals and has participated on numerous U.S. EPA peer-review panels. His primary interests are in air pollution and global climate effects to terrestrial ecosystems; native plant community responses (shifts in diversity) to air pollutants and global climate change; plant-stress-air pollution/global climate change interactions; air toxics; and urban forestry.

Dr. Jack Fishman has been studying ozone for more than 30 years. He came to NASA in 1979 where he has developed a way to measure ozone pollution from satellites. Using satellites, he discovered large plumes of ozone pollution coming from industrialized regions of the world such as the eastern United States, Europe, and eastern Asia. Somewhat surprisingly, he also found large amounts of ozone over the South Atlantic Ocean. Such plumes could even be identified in measurements thousands of kilometers from their origin. In 1992, he led a group of scientists to investigate the composition and origin of these elevated ozone concentrations where they flew in NASA's specially instrumented DC-8 airplane over a 5-week period. The source of the pollution was found to be widespread biomass burning in southern Africa and Brazil and a unique meteorological situation that resulted in both plumes being transported over ocean areas adjacent to Angola and Namibia. In 1990, Dr. Fishman co-authored Global Alert: The Ozone Pollution Crisis, a book for general audiences that expressed how important the issue of global pollution is and how measurements may be taken to reduce the detrimental effects of widespread ozone pollution.

Dr. Irene Ladd has been involved in educational reform both locally and statewide for the past 25 years. She is a retired teacher of 33 years and joined the Surface Ozone Measurement for GLOBE (SMOG) Team to develop the surface ozone protocol and field test the instruments used for measuring surface ozone. She has developed a training program and educational materials to integrate the study of surface ozone with core curricula. Dr. Ladd continues to provide national and international training and guidance to implement inquiry in the classroom through authentic science and student research. The collaboration to incorporate the use of ozone sensitive plants with the Surface Ozone Protocol is a natural extension to taking surface ozone measurements. Dr. Ladd was educated at Keene State College, University of New Hampshire, and Vanderbilt University, Tennessee.

Dr. Howard Neufeld received his B.S. in Forestry from Rutgers University in 1975, his M.F. in Forest Sciences from the Yale School of Forestry and Environmental Science in 1977, and his Ph.D. in Botany from the University of Georgia in 1984. Currently he is a professor of Biology at Appalachian State University, Past-President of The Association of Southeastern Biologists (ASB), and President-Elect of the Southern Appalachian Botanical Society. His research expertise is in the area of plant physiological ecology and has included work on plants in swamps, deserts, and forest understories. For the past 20 years, he has been active in air pollution effects research, including acidic deposition (rain and fog) studies on spruce trees and hardwoods of the eastern United States, and tropospheric ozone on native wildflowers. From 1988-1992 he was the principal investigator of a National Park-U.S. EPA sponsored research project on the effects of ozone on plants native to Great Smoky Mountains National Park. These results have been published in a variety of journals, and additional papers are in preparation and press.

Dr. Margaret Pippin is a atmospheric scientist in the Science Directorate at NASA's Langley Research Center. She came to Langley in 2001 after completing post-doctoral research at Western Michigan University, where she specialized in making measurements of organic nitrates at the University of Michigan Biological Station. She comes from a background of both modeling and field measurements, with an emphasis on data analysis. She is interested in the analysis of observational data sets to better understand the chemistry of the atmosphere with a particular interest in the chemistry of biogenic hydrocarbons and their ozone production potential. Dr Pippin has been active in science education for over 20 years and enjoys working with students of all ages.

Dr. Pippin has spent the past several years improving the quality of the GLOBE surface ozone measurements. She has performed extensive laboratory testing to determine the relationship between the absorbance (color change) of the Eco-Badge test cards and the ozone concentration, and the dependence of this color change upon temperature and humidity. Dr. Pippin was involved in GLOBE teacher training for the Surface Ozone Protocol and often visits GLOBE schools to discuss atmospheric science and methods of analysis with the students.

Susan Sachs is a National Park Ranger and the education coordinator of the Appalachian Highlands Science Learning Center located in Great Smoky Mountains National Park. A principal part of her job involves creating educational opportunities from the research that occurs in the parks of the Appalachian Highlands monitoring network (besides the Smoky's network she covers Big South Fork National Recreation Area, Obed Wild and Scenic River and the Blue Ridge Parkway). Her personal education philosophy is that people learn best when they are involved in learning activities that are both meaningful and relevant so many of the education programs at her center involve students, teachers and others in collecting data for actual research projects. The ozone biomonitoring project is one example of several citizen science projects on going at the Appalachian Highlands Science Learning Center. Susan was educated at the University of Maryland, College Park and has lived and worked in National Parks in Washington, DC, Alaska, Arizona, and California. Ms Sachs currently resides in the biologically diverse mountains of North Carolina.

Dr. John M. Skelly is Professor Emeritus of Plant Pathology from the Department of Plant Pathology, Pennsylvania State University. He holds a BS in Forestry 1962, MS 1964; and Ph.D. 1968 in Plant Pathology from Penn State. He taught at Virginia Polytechnic Institute and State University 1968-1982 and returned to Penn State 1982-2004. His expertise is in forest pathology with specialization in air pollution-caused effects to forest trees and native plants within North Temperate regions. Skelly has been involved in writing the Criteria Documents for the U.S. EPA and as a consultant to the U.S. Dept. of Justice and the United Nations on diagnosing air pollution caused injury to plants. International cooperative projects have been held with colleagues in Canada, Mexico, Switzerland, Spain, Germany, and Italy.

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